

**THE MODULATING EFFECTS OF PHYSIOLOGICAL, GENETIC, AND
BIOCHEMICAL FACTORS ON THE SEQUELAE OF
CHILDHOOD TRAUMATIC BRAIN INJURY**

Tsz-YAN M. Lo

PhD

THE UNIVERSITY OF EDINBURGH

YEAR OF PRESENTATION: 2009

DECLARATION

I, Tsz-Yan M. Lo, declare:

- (a) that the thesis has been composed by myself, and
- (b) that as a member of the Edinburgh-Newcastle Paediatric Traumatic Brain Injury Research Group, I have made a substantial contribution to the work and I have disclosed within the thesis of my contributions and the relative contributions of the respective workers or authors where the work is based on joint research or publications, and
- (c) that the work has not been submitted for any other degree or professional qualification.

Dr. Tsz-Yan M. Lo 11 May 2009

ABSTRACT

Brain trauma occurs frequently in children and its consequences cause significant health and financial burden to the patients, their carers and society. This thesis assessed the modulating effects of physiological, genetic, and biochemical factors on the sequelae of childhood brain trauma.

Primary brain injury from the mechanical forces of trauma and secondary brain insults consequent on the primary injury are determinants of brain trauma outcome. The most important secondary insults recognised are reduced cerebral perfusion pressure (CPP) and raised intracranial pressure (ICP). CPP is governed by the mean arterial blood pressure and the ICP. During childhood these physiological measures change with age. With continuous physiological recordings, 'critical' age-related minimum CPP thresholds for children aged 2-6, 7-10 and 11-15 years were defined as 48, 54 and 58mmHg respectively. Utilising these thresholds and a novel cumulative pressure-time index (PTIc), we have demonstrated that CPP insult still remains a feature in 80% of the severe brain trauma patients and significantly relates to global outcome.

Brain trauma and cerebral ischaemia are stimuli to the inflammatory cascade leading to further brain damage. Serum adhesion molecule levels after brain trauma indicate injury severity and predict outcome better than brain specific proteins. Predictability is improved using more than one serum biomarker level. Neuro-inflammatory pathways involving adhesion molecules may have a strong modulating effect on brain trauma outcome but warrants further investigations in relation to CPP insult.

Genetic factors such as Apolipoprotein E (APO E) genetic polymorphisms may additionally influence outcome, but it was not known whether genetic factors lessen the quantity of CPP insult or the cellular response to it. We demonstrated that the e4 carriers who had unfavourable outcome had 22 times less CPP insult than the non-e4 carriers, while the e3 homozygous who had good recovery had 26 times more CPP insult than the non-e3 homozygous. This suggests that APO E polymorphisms may affect the patient's cerebral ischaemic tolerance differently, indicating especially the need to prevent CPP insult among e4 carriers.

Cerebral ischaemia may, therefore, be a common pathway through which physiological and genetic factors modulate outcome after brain trauma.

CONTENT

CHAPTER 1:	
OVERVIEW OF CHILDHOOD TRAUMATIC BRAIN INJURY (TBI)	1
1.1) Introduction	1
1.1.1) Terminology Definition	2
1.2) Epidemiology	3
1.2.1) Epidemiology of Childhood TBI in Scotland	3
1.2.2) Difficulties in Comparing Childhood TBI Epidemiology Data	5
1.2.3) Comparison between International and Scottish Childhood TBI Epidemiology	7
1.2.4) Causes of Paediatric TBI	9
1.2.5) Classification of Brain Injury Severity	10
1.2.5.1) Coma Scales	10
1.2.5.2) Other System Injury Score	15
1.2.5.2.1) Injury Severity Score (ISS)	16
1.2.5.2.2) Revised Trauma Score (RTS)	16
1.2.5.2.3) Trauma Score-Injury Severity Score (TRISS)	17
1.2.5.2.4) Paediatric Trauma Score (PTS)	17
1.2.6) Outcome	17
1.2.6.1) Global Outcome Assessment	17
1.2.6.2) Mortality	20
1.2.6.3) Morbidity	20
1.3) Determinants of Outcome after Brain Trauma	22
1.3.1) Introducing Primary Brain Injury and Secondary Brain Insult	22
1.3.2) Secondary Brain Insults	23
1.3.2.1) Secondary Physiological Derangements	23
1.3.2.1.1) Frequency of Secondary Physiological Derangement after Brain Trauma	24
1.3.2.1.2) Secondary Physiological Derangement and Brain Trauma Outcome	25
1.3.2.2) Critical Care Neuro-trauma Management	25
1.3.2.2.1) Lack of Normative Age-related Intracranial Physiological Data	28
1.3.2.2.2) Limitation in Research Methodology to Quantify Physiological Derangements	29
1.3.2.3) Cellular and Molecular Pathophysiological Response to Brain Trauma	30
1.3.2.3.1) Secondary Brain Injuring Mechanisms	31
1.3.2.3.1.1) Hypoxic / Ischaemic Injury	31
1.3.2.3.1.2) Excitotoxicity	34
1.3.2.3.1.3) Oxidative Stress	35
1.3.2.3.1.4) Neuro-inflammation	35

1.3.2.3.1.5) Apoptosis	38
1.3.3) Genetic Factors	39
1.4) Neuropathology in Fatal Childhood Brain Trauma	40
1.5) Outcome Prediction During the Acute Phase Post Brain Trauma	41
1.6) Summary	43
1.7) Hypotheses & Aims	45
 CHAPTER 2:	
CRITICAL AGE-RELATED CEREBRAL PERFUSION PRESSURE THRESHOLDS (CPP) & QUANTIFICATION OF THE TOTAL BURDEN OF CPP INSULT	48
2.1) Introduction	48
2.2) Hypotheses and Aims	51
2.3) Patients and Methods	52
2.3.1) Design of the Study	52
2.3.2) Patients	53
2.3.3) Data Collections and Analyses	55
2.3.3.1) Physiological Data Collection	55
2.3.3.1.1) Original Data Collection Plan	55
2.3.3.1.2) Actual Data Collection Methods	56
2.3.3.1.3) Physiological Parameters Measured	57
2.3.3.2) Outcome Data Collection	58
2.3.3.3) Data Analyses	59
2.3.3.3.1) Choosing Age-Specific Thresholds (Age Index)	59
2.3.3.3.1.1) Heart Rate	59
2.3.3.3.1.2) Hypoxia	60
2.3.3.3.1.3) Pyrexia	60
2.3.3.3.1.4) Arterial Blood Pressure	60
2.3.3.3.1.5) Intracranial Pressure (ICP)	61
2.3.3.3.1.6) Cerebral Perfusion Pressure (CPP)	63
2.3.3.3.2) Physiological Data Validation and Analyses	64
2.3.3.3.3) Logistic Regression Modelling & Discrimination Assessments - Misclassification Rate & Receiver-Operator Characteristic (ROC) Curves	65
2.3.3.3.4) Derivation of the Cumulative Pressure-Time Index (PTI)	66
2.3.3.3.4.1) Calculation of the PTI for CPP (PTIc)	68
2.3.3.3.4.1.1) Allocation of Age Groups	68

2.3.3.3.4.1.2) Determination of the Age Band Related Critical CPP Thresholds	69
2.3.3.3.4.2) Calculation of the PTI for ICP (PTI _i)	69
2.4) Results	70
2.4.1) Current Cohort	70
2.4.1.1) Physiological Data	71
2.4.1.2) Comparison with Pilot Group	71
2.4.2) Combined Current and Pilot Group Data Set	72
2.4.3) Determining the Best Predictors of Outcome	73
2.4.3.1) Those with ICP/CPP Monitoring	73
2.4.3.1.1) Alive vs. Dead	74
2.4.3.1.2) Poor vs. Independent Outcome	75
2.4.4) PTI Analyses	76
2.4.4.1) PTI for CPP	77
2.4.4.2) PTI for ICP	82
2.5) Discussions	86
2.5.1) Physiological Derangement	86
2.5.1.1) CPP and ICP Derangement	88
2.5.1.1.1) CPP Derangement	88
2.5.1.1.1.1) Incidence and Duration	88
2.5.1.1.1.2) Causes of CPP Derangement	90
2.5.1.1.2) ICP Derangement	92
2.5.1.1.3) Quantification of the Total Burden of Secondary Intracranial Physiological Insult	95
2.5.1.1.3.1) PTI for CPP (PTI _c)	96
2.5.1.1.3.2) PTI for ICP (PTI _i)	98
2.5.1.1.3.3) Critical Thresholds	99
2.5.1.2) Other Derangement	101
2.5.1.2.1) Blood Pressure Derangement	101
2.5.1.2.1.1) Hypotension	101
2.5.1.2.1.2) Hypertension	103
2.5.1.2.2) Hypoxia	105
2.6) Monitoring of Brain Oxygen & Substrates Delivery & Consumption	110
2.6.1) Brain Tissue Oxygen Delivery & CO ₂ Removal Monitoring	110
2.6.2) Monitoring of Cerebral Blood Flow & Metabolisms in the Injured Brain	111
2.6.2.1) Jugular Venous Bulb Oximetry	112
2.6.2.2) Transcranial Doppler Ultrasound (TCD)	114
2.6.2.3) Microdialysis	115
2.7) Limitations	117
2.8) Conclusions	120

CHAPTER 3:	
VARIABILITY AND INTER-RELATIONSHIPS OF ICP, CPP, AND MAP SIGNALS AND THEIR RELATIONSHIPS WITH OUTCOME AFTER CHILDHOOD TBI	121
3.1) Introduction	121
3.2) Aims	122
3.3) Patients and Methods	122
3.3.1) Design of the Study	122
3.3.2) Patients	122
3.3.3) Data Collections	123
3.3.3.1) Demographic Data	123
3.3.3.2) Physiological Data	124
3.3.3.3) Outcome Data	125
3.3.4) Analyses	125
3.4) Results	125
3.4.1) Demographic Results	125
3.4.2) Variability	127
3.4.2.1) Total Duration	128
3.4.2.2) 1 st Epoch (First 48 hours from the Time of Injury)	128
3.4.2.3) 2 nd Epoch (48 – 96 hours from the Time of Injury)	128
3.4.2.4) 3 rd Epoch (96 – 144 hours from the Time of Injury)	129
3.4.3) Patterns	129
3.4.3.1) Descriptions of Patterns	129
3.4.3.2) Patterns in Relation to Outcome Scores	133
3.4.3.3) Patterns in Relation to Fatal Cases	133
3.5) Discussions	134
3.5.1) Variability	136
3.5.2) Pattern Types	138
3.6) Conclusions	140
 CHAPTER 4:	
MODULATING EFFECT OF APOLIPOPROTEIN E POLYMORPHISMS ON SECONDARY INSULT AND OUTCOME AFTER CHILDHOOD BRAIN TRAUMA	141
4.1) Background	141
4.2) Aims	143
4.3) Patients and Methods	144
4.3.1) Design of the Study	144

4.3.2) Patients	144
4.3.3) Controls	144
4.3.4) Sample and Data Collection	145
4.3.5) Outcome Assessment	145
4.3.6) Laboratory Methodology	146
4.3.6.1) APO E Genotyping	146
4.3.6.1.1) DNA Extraction from One Buccal Brush	146
4.3.6.1.1.1) Buccal Smear Sample Collection	146
4.3.6.1.1.2) Cell Lysis	147
4.3.6.1.1.3) RNase Treatment	147
4.3.6.1.1.4) Protein Precipitation	147
4.3.6.1.1.5) DNA Precipitation	148
4.3.6.1.1.6) DNA Hydration	149
4.3.6.1.2) Polymerase Chain Reaction (PCR)	149
4.3.6.1.2.1) PCR Mastermix Components	150
4.3.6.1.2.2) PCR Programme	151
4.3.6.1.2.3) Detection of PCR Product using Gel Electrophoresis	151
4.3.6.1.2.3.1) 3% Agarose Gel	152
4.3.6.1.2.3.2) TBE Buffer	152
4.3.6.1.2.3.3) Gel Loading Buffer (Blue) for Agarose Gel	153
4.3.6.1.2.3.4) 1kb Ladder Marker	153
4.3.6.1.3) Restriction Enzyme Digest on PCR Products	153
4.3.6.1.4) APO E Genotype Identification Using Gel Electrophoresis	154
4.3.6.1.4.1) 4% Metaphor Agarose Gel	155
4.3.6.1.4.2) Gel Loading Buffer (Orange) for Metaphor Agarose Gel	156
4.3.6.1.4.3) 25 kb Ladder Marker	156
4.3.7) Data Analyses	156
4.3.7.1) APO E Allelic Distributions	156
4.3.7.2) Allelic Dichotomies and Outcome Groups	157
4.3.7.3) APO E Alleles and Outcome	157
4.3.7.4) APO E Alleles, Demographic Details, Injury Severity, and Secondary Insults	158
4.3.7.4.1) APO E Alleles and Age-Related CPP Insult	158
4.3.7.5) APO E Alleles, Demographic Details, Age-Related CPP Insult, and Outcome	158
4.4) Results	160
4.4.1) Demographics	160
4.4.2) APO E Allelic Distributions	161
4.4.3) APO E Alleles and Outcome	161
4.4.4) APO E Alleles and Secondary Derangement	163
4.4.4.1) APO E Alleles and the Total Burden of CPP Insult	163
4.4.5) APO E Alleles, CPP Insult, and Outcome	164
4.4.5.1) At PICU Discharge	164

4.4.5.2)	6 Months Post Injury	166
4.4.6)	Determinants of Outcome after Childhood Brain Trauma	167
4.4.6.1)	Delayed Returned of Consciousness at PICU Discharge	167
4.4.6.2)	Poor Outcome at 6 Months Post Injury	168
4.5)	Discussions	169
4.5.1)	Over-Representation of the e2 Allele in Active Children with or without Brain Injury	171
4.5.2)	Different Amount of CPP Insult between the Carriers of the Different APO E Alleles	173
4.5.3)	APO E Polymorphisms Influence Recovery at Different Time Points Post Injury	176
4.5.4)	Limitations of the Study	178
4.6)	Conclusions	179
 CHAPTER 5:		
BRAIN TRAUMA SERUM BIOMARKERS AND THEIR PROGNOSTIC VALUES FOR UNFAVOURABLE OUTCOME AFTER BRAIN TRAUMA		180
5.1)	Background	180
5.1.1)	Brain Specific Proteins	181
5.1.2)	Inflammatory Mediators	185
5.1.2.1)	Cytokines in Acute Brain Injury	185
5.1.2.1.1)	Interleukins 6 (IL-6)	186
5.1.2.1.2)	Interleukins 8 (IL-8)	187
5.1.2.1.3)	Interleukins 10 (IL-10)	188
5.1.2.2)	Adhesion Molecules	190
5.1.3)	Vasoconstrictor (Endothelins)	191
5.1.4)	Difficulties in Development of Neurochemical Monitoring	192
5.2)	Aims	193
5.3)	Patients and Methods	193
5.3.1)	Sample Collections	194
5.3.2)	Marker Assays	195
5.3.2.1)	Basic Principles of Sandwich Enzyme Linked Immuno-sorbent Assays	196
5.3.3)	Statistical Analyses	197
5.3.3.1)	Receiver-Operator Characteristic (ROC) Curve Analyses	198
5.3.3.2)	Multivariate Receiver-Operator Characteristic (MultiROC) Curve Analyses	198
5.3.3.2.1)	Defining Prognostic Thresholds for Screening Marker in the Prognostic Rules	198
5.3.3.2.2)	Defining Prognostic Rules for MultiROC Analyses	199

5.3.3.2.3) Assessment of the Predictive Values of the Prognostic Rules	200
5.4) Results	201
5.4.1) Injury Severity and Outcome	201
5.4.2) Mediator Concentrations and Injury Severity	202
5.4.3) Mediator Concentrations and Initial Brain CT Findings	202
5.4.4) Mediator Concentrations and Outcome	203
5.4.4.1) At PICU Discharge	203
5.4.4.2) 6 Months Post Injury	204
5.4.5) Comparison of the Outcome Prediction Performance between Different Prognostic Rules and Individual Marker Levels	206
5.5) Discussions	209
5.5.1) Investigating the Diagnostic and Predictive Values of Multiple Biomarkers of Different Mediator Families	209
5.5.2) Biomarkers and Indication of Injury Severity	210
5.5.3) Biomarkers and Prediction of PICU Discharge Coma Status	212
5.5.4) Biomarkers and Outcome Prediction	213
5.5.4.1) Brain Trauma Outcome Prediction with Glial and Neuronal Proteins	213
5.5.4.2) Brain Trauma Outcome Prediction with Interleukins	214
5.5.4.3) Brain Trauma Outcome Prediction with Adhesion Molecules	215
5.5.4.4) L-Selectin and IL-8 Serum Levels had the Highest Outcome Predictive Values	215
5.5.4.5) Brain Trauma Outcome Prediction with Prognostic Rules Combining 2 Biomarker Levels	217
5.5.4.5.1) Multivariate Receiver-Operator Characteristic (MultiROC) Curve Analyses	217
5.5.4.5.2) Screening and Varying Markers in Prognostic Rules and Their Effects on the Rule's Prognostic Performance	218
5.5.5) Limitations of the Study	220
5.6) Conclusions	220
 CHAPTER 6:	
NEUROPATHOLOGICAL FEATURES OF FATAL PAEDIATRIC TBI	222
 6.1) Background	222
 6.2) Hypotheses and Aims	226

6.3) Methods	226
6.3.1) Original Proposed Methods	226
6.3.1.1) Problems Encountered During Recruitment	227
6.3.2) Actual Study Methods	228
6.4) Results	230
6.4.1) Mode of Brain Trauma and Survival Durations	230
6.4.2) Distributions of the Different Pathological Findings	230
6.4.3) Comparisons of the Neuropathological Features Between Different Survival Durations	232
6.4.3.1) Survival Less than 24 Hours vs. Survival of 24 Hours or More Post Injury	232
6.4.3.2) Instantaneous Deaths vs. Non-instantaneous Deaths	233
6.4.4) Comparisons of the Neuropathological Features Between Different Modes of Brain Injury	234
6.4.4.1) Road Traffic Accidents (RTA) vs. Non-RTA	234
6.4.4.2) Accidental Head Injuries vs. Non-Accidental Head Injury (NAHI)	235
6.4.4.3) Falls vs. Non-Falls	236
6.5) Discussions	237
6.5.1) Diffuse Brain Swelling	238
6.5.2) Hypoxic Ischaemic Damage	239
6.5.3) Diffuse Axonal Injury	240
6.5.4) Limitations	247
6.6) Conclusions	248
 CHAPTER 7:	
MODULATING EFFECTS OF PHYSIOLOGICAL, GENETIC, AND BIOCHEMICAL FACTORS ON THE SEQUALAE OF CHILDHOOD BRAIN TRAUMA	250
7.1) Introduction	250
7.2) Cerebral Ischaemia and Brain Trauma Recovery	251
7.3) Potential Modulating Mechanisms of APO E Genetic Polymorphisms on Brain Trauma Outcome	255
7.3.1) Potential Mechanisms Assessed by Clinical or Post-mortem Studies in the Literature	255
7.3.1.1) Apolipoprotein E Polymorphisms and Coagulation	255
7.3.1.2) APO E Genotypes and Post-Traumatic Cerebral Swelling	258
7.3.1.3) APO E Genotypes and the Conversion of the β Amyloid Precursor Protein to β Amyloid	259

7.3.1.4) Limitation of Post-Mortem Studies for Assessing Potential Mechanisms of Action	260
7.3.2) Potential Mechanisms Assessed by Animal or In-Vitro Studies	260
7.3.3) APO E Genetic Polymorphisms and Cerebral Ischaemic Tolerance: Evidence from Bench and Bedside Studies	262
7.4) Brain Trauma Biomarker and Outcome	263
7.5) Ethical Considerations	267
7.5.1) Consent to Participate in Research and Critically Ill Children	268
7.5.2) Ethical Considerations in Research Involving Human Tissues	270
7.5.2.1) Organ Retention Scandals – The Alder Hey Inquiry	270
7.5.2.2) Impact of the Alder Hey Inquiry on Post-Mortem Rate, Manpower and Training in Histopathology	272
7.5.2.3) Impact of the Alder Hey Inquiry on Research	273
7.5.2.4) Post-Mortem and Research in the Future	274
7.6) Limitations	275
7.7) Summary	275
 CHAPTER 8:	
CONCLUSIONS AND FUTURE RESEARCH PROPOSAL	277
 8.1) Thesis Conclusions	277
8.1.1) Summary of Significant Findings	277
8.1.2) Conclusions	278
 8.2) Future Research Proposal	279
8.2.1) Future Research Proposal Introduction	279
8.2.2) Hypotheses	282
8.2.3) Aims and Objectives	282
8.2.4) Proposed Methodology	283
 BIBLIOGRAPHY	285
 LIST OF ABBREVIATIONS	325
 ACKNOWLEDGEMENTS	326

APPENDICES	327
Appendix I: Age Index for Physiological Derangements	328
Appendix II: Details of the Neurochemical Marker Assay Procedures	329
Appendix III: Presentations	351
Appendix III-a) Presentation Summary Lists	352
Appendix III-b) Sample Presentations	355
Appendix IV: Publications	362
Appendix IV-a) Publication Summary Lists	363
Appendix IV-b) Published Papers	366

CHAPTER 1: OVERVIEW OF CHILDHOOD TRAUMATIC BRAIN INJURY (TBI)

1.1) INTRODUCTION

Traumatic brain injury (TBI), from either accidental or non-accidental origin, occurs frequently in children and is responsible for up-to 50% of all A&E attendants less than 15 years of age [1]. Despite major advances over the recent years in resuscitation and trauma care, brain trauma remains the commonest cause of trauma death and long-term disability in children [2, 3] . For every 100,000 population in Scotland, there are more than 100 new cases of disability occurring each year as a result of brain trauma [4] . The consequences can be profound neurological deficits [5] including severe developmental delay if the injury is sustained in early childhood, resulting in an enormous burden of caring for parents, carers, and society.

Intensive care management of critically ill brain injured children remains largely empirical [6] because the paucity of clinical research in this area has translated into only limited advances in certain specific areas of monitoring and treatment. The majority of animal models for brain injury have been small rodents, whose anatomy and physiology are very different from those of humans, and these experiments often fail to consider the effects of neuro-intensive care. As a result, data from these models have limited potential for extrapolation to intensive care treatments. Similarly, most basic animal research has employed adult animal models with few studies examining animals equivalent to the newborn infant or young child. Clinical studies investigating the complex pathophysiological processes initiated by brain

trauma in critically ill infants and children and experimental studies using immature animals are, therefore, required urgently before evidence based recommendations and guidelines can be developed rationally for the intensive care management of the brain injured infants and children.

An overview of the childhood traumatic brain injury literature is presented and is followed by formulation of the hypotheses to be investigated in this thesis.

1.1.1) Terminology Definition

Head injury may be defined as injuries to the scalp, skull, or brain caused by an external force applied to the head from either accidental or non-accidental means i.e. trauma to the head. The extent of the structural damage to the head following trauma depends upon the intensity and severity of the mechanical insult. It is, therefore, possible to have sustained a head injury isolated to the scalp without involvement of the underlying skull or brain. Head injury is, therefore, not synonymous to brain injury, which by definition, signifies injuries to the brain.

Brain injury may be the result of congenital or acquired insults. Acquired brain injury may be caused by trauma, neoplasm, infections, inflammation, degeneration, autoimmune reactions, thrombosis, or bleeding.

The patients studied in this thesis have suffered brain injury from trauma and for consistency sake they will be referred to as having sustained traumatic brain injury (TBI) or brain trauma.

1.2) EPIDEMIOLOGY

1.2.1) Epidemiology of Childhood TBI in Scotland

The epidemiology of childhood traumatic brain injury in Scotland has only been described previously in four reports which are summarised below.

The hospital admission rate for childhood head injury in Scotland between 1984 and 1985 was 4011 per 100 000 children per year which was determined in a retrospective case review involving all attending children aged 14 years or less with head injury of all severity among 23 different Scottish A&E departments [7]. Brookes and colleagues then identified those with evidence of brain injury within the cohorts (i.e. children who had altered consciousness on arrival to hospital or had a history of altered consciousness and amnesia on arrival to the A&E department) to calculate the hospital admission rate for childhood traumatic brain injury which was 290 per 100 000 children per year [7]. Majority of these brain injured children had suffered a mild injury but five to ten percents of the patients had sustained a severe injury giving the incidence of severe paediatric traumatic brain injury to be between 14.5 and 29 per 100 000 children per year [7]. Road traffic accidents were the cause

of severe brain injury in 75% of cases and responsible for 71% of brain trauma death [7].

Jennett and co-workers subsequently investigated the A&E attendants in Scotland during 1985 [1]. This retrospective study reported similar incidence for childhood head injury and childhood traumatic brain injury to those reported by Brookes and co-workers [7] which was not surprising given the same catchment population was used for both reports and the time lapse between the studies was insufficient to demonstrate any effect from injury prevention measures. Childhood brain trauma mortality rate in Scotland was reported for the first time in this study and was thought to be 5.3 per 100 000 children per year [1].

Scottish childhood injury mortality rate between the years of 1981 and 1985 was reported by Morrison's group in 1999 to be 11.6 per 100 000 children per year [8]. This figure was based upon retrospective data collection from Registrar General and included infants and children aged between 0 and 14 years [8]. Similar to the two previous Scottish reports, road traffic accident related mortality rate was high (4.8 per 100 000 children per year) which was eight times more than the mortality rate related to accidental falls (0.6 per 100 000 children per year) [8].

National mortality data collected by Registrar General in Scotland over a ten-year period (1986 to 1995) was reviewed by Williamson and colleagues [9]. A total of 290 children died as a result of traumatic brain injury during the study period giving a mean annual head injury mortality rate of 3 per 100 000 children [9]. This study

reported a 2.3 times reduction in paediatric head injury mortality rate over ten years (4.1 per 100 000 children in 1986 vs. 1.8 per 100 000 children in 1995) [9] which may be the result of the various injury prevention measures introduced during the study period. This report also highlighted the existence of significant variations in childhood brain trauma mortality between socially deprived and affluent areas in Scotland with the highest paediatric head injury mortality rate (5.1 per 100 000 children) observed in the most deprived area [9]. This was 2.4 times higher than the childhood head injury mortality rate observed in the most affluent area (2.1 per 100 000 children) [9]. Similar to previous reports, pedestrian accidents were the most frequent cause of paediatric head injury mortality.

1.2.2) Difficulties in Comparing Childhood TBI Epidemiological Data

The incidence of childhood brain trauma relies upon a few existing epidemiological studies which often involved different patient populations such as A&E attendants, patients admitted for neuro-observations, patients with evidence of brain injury, or critically ill brain trauma patients. Comparison between studies is, therefore, difficult. Attempts to compare the 4 available Scottish childhood brain trauma epidemiology reports in the literature clearly demonstrate this difficulty. Hospital admission rate was used in Brookes and Jennett's papers [1, 7] while injury mortality rate and head injury mortality were reported in Morrison and Williamson's groups [8, 9] respectively. Two of the four Scottish studies investigated the mortality data collected by Registrar General without considering survivors of paediatric brain

trauma [8, 9], while the authors of the remaining papers reported data drawn from A&E attendants [1, 7].

Inter-hospital transfers of brain trauma patients create another problem for reliable prevalence comparison between studies because with the danger of ‘double counting’, it is difficult to ascertain the accurate number of brain trauma patients and to define catchment populations. As a result, many reports of brain trauma patient numbers and fatalities from trauma units or pathology departments do not usually provide a population denominator.

Variations in admission and triage policies between hospitals also make comparison difficult between reports with apparently similar patient groups. The 9th edition of the International Classification of Diseases (ICD) is the most widely used coding system in the UK, but there are 10 non-mutually exclusive codes in the ICD to cover the diagnosis of head injury. This makes accurate identification of head injury patients difficult and may increase the risk of double counting. Application of these codes may also be different between hospitals and countries making the validity of the comparison between reports in the literature doubtful. In addition, the ICD codes are not capable of differentiating injury severity and this makes retrospective identification of severe injury impossible from ICD codes alone without reference to impairment of conscious levels. Finally coding errors occur frequently and may not be easily identifiable.

1.2.3) Comparisons Between the International And Scottish Childhood TBI Epidemiology

In America, a steady decline in mild and moderate childhood head injury has been observed over the past 3 decades but the incidence of severe paediatric brain trauma was slightly increased. The mean paediatric brain trauma incidence during the period between 1930s and 1980s was between 180 and 310 per 100 000 per year [10-12], with a childhood brain trauma mortality rate of around 8 per 100 000 per year [10-12]. By the late 1980s and 1990s, the mean incidence of childhood brain trauma has reduced to between 60 and 160 per 100 000 per year [13-16], and the mortality rate was between 5 and 6 per 100 000 per year [13-17]. Brain trauma incidence was consistently higher in the rural areas than metropolitan regions [13-18], and it was 3 times more common in boys than girls [10, 13, 18]. Falls was the main cause of brain trauma in children aged between 0 and 4 years of age while motor vehicle related accidents were the commonest cause of brain injury among children older than 4 years of age [14, 15, 17-21].

Epidemiological data from European countries often only included severe brain injury and would, therefore, appear less than those reported by US authors since many US reports included head injury of all severity. If only severe brain injury was considered, the incidence was fairly similar between the reported European and American figures.

During the 1980s and 1990s, the mean incidence of childhood severe brain trauma in Northern European countries (Finland, Denmark, Iceland, and Sweden) was 12 – 17 per 100 000 per year while the annual mortality rate was 2.6 – 4.8 per 100 000 [3, 22-24]. In 1996, the mean incidence of childhood severe brain trauma in France [25] was reported to be lower (8.4 per 100 000 per year) than those reported by the Northern European countries [3, 22-24]. Similarly, the mortality rate was also lower and was found to be between 1.3 and 2.1 per 100 000 per year [25]. However, the mean incidence of severe paediatric brain trauma in Switzerland was as high as 15.2 per 100 000 per year and the mortality rate was 6.8 per 100 000 children [26]. Motor vehicle related accidents were the main cause of childhood brain trauma in Europe [3, 25, 27]. The European annual incidence of severe childhood brain trauma is, therefore, between 8 and 18 per 100 000 children with a mortality rate between 2 and 7 per 100 000 children.

Given the aforementioned limitations to the comparability between reports, the hospital admission rate for head injury in Scotland [1, 7] is similar to those reported in the literature for the United States of America and other European countries [3, 13-26] which were between 100 to 300 per 100 000 children per year. Little difference exists in the reported proportion of severe brain injury in children across the world [1, 3, 7-9, 13-26] giving an estimated incidence of 10 – 30 per 100 000 children per year for severe brain injury in children.

1.2.4) Causes of Paediatric TBI

Accidental fall remains the commonest cause of childhood traumatic brain injury worldwide but when only severe brain injury is considered, motor vehicle accident related injuries become the commonest cause of brain trauma in developed countries [1, 3, 7-9, 14, 15, 17-21, 25, 27]. Different road users, for examples pedestrian and motor vehicle occupants, may become victims of brain injury from motor vehicle accidents. Significant variations exist worldwide in whom the commonest brain injury victims are in motor vehicle related accidents [1, 3, 7-9, 14, 15, 17-21, 25, 27]. Pedestrian is the commonest type of road users to sustain brain injury from motor vehicle related accidents in Britain [1, 7-9], while motor vehicle occupants constitute the majority of brain injury sufferers following such accidents in the Untied States of America [14, 15, 17-21]. This variation reflects the different road and vehicle usage between countries and needs to be taken into consideration when designing local accident preventative measures.

Sample statistics of A & E attendants with head injury at Royal Hospital for Sick Children in Edinburgh over a 1-year period demonstrated a similar pattern with accidental falls accounting for half (53%) of these attendances and 12% had a history of significant falls (i.e. more than 1 meter height). Motor vehicle related accidents were responsible for 4.6% of attendances and the remainder (42%) were due to miscellaneous causes including non-accidental head injury. Similar to other reported series, toddlers i.e. those between one and two years of age represented the peak age

group of attendants with a steady decline in attendance thereafter up to 13 years of age.

1.2.5) Classifications of Brain Injury Severity

Severity of brain injury may be classified using the post resuscitation Glasgow Coma Scale (GCS) [28], duration of unconsciousness or post-traumatic amnesia, and neuro-imaging scales reserved for severe injury e.g. Marshall Scale [29, 30]. Coma or loss of consciousness is caused by primary midbrain injury from rotation of the cerebral hemispheres on the midbrain. Since rotational forces are not necessary the cause of all head trauma, not all head injury are associated with a loss of consciousness (e.g. some frontal lobe injuries, extradural haematomas and some penetrating and compression injuries), but conversely, all cases with loss of consciousness indicate brain injury. Jennett [1] considers coma to be clinical evidence of damage to the brain and the most reliable way to recognise that a brain injury has occurred. Thus, evidence of brain injury is indicated by an altered consciousness on arrival or a history of altered consciousness with amnesia on arrival, or abnormal neurological signs.

1.2.5.1) Coma Scales

The GCS (Table 1.1a) [28], which is essentially a mini neurological examination, is the most frequently used method to classify injury severity in adult brain trauma. It enables comparisons between successive examinations of the consciousness level to

identify any clinical deterioration. GCS also remains the most useful clinical index of brain injury because it provides a gradient of injury severity: a summated score of 13 – 15 is designated mild, a score of 9 – 12 is designated moderate, and a score of 3 – 8 is designated severe injuries. To minimise the subjectivity of GCS in the classification of brain injury severity, the British Society of Rehabilitation Medicine has elected to use a combination of GCS and duration of unconsciousness to classify head injury severity: mild injury is defined as an injury causing unconsciousness for less than 15 minutes and a post-resuscitation GCS of 13 to 15; moderate injury is defined as any injury causing loss of consciousness for more than 15 minutes and a post-resuscitation GCS of 9 to 12; and severe injury is defined as any injury causing unconsciousness for more than 6 hours and a post-resuscitation GCS of 3 to 8. However, the criteria used in this classification system are not mutually exclusive and may pose difficulty when, for example, a patient presents with a post-resuscitation GCS of 4 but was only unconscious for 3 hours.

Many of the responses assessed in the GCS require an adult level of neurocognitive function and cannot be easily graded in children less than 10 years of age [31]. Modifications of the GCS to include age-adjusted verbal and motor responses have been described and used to assign severity for childhood brain injuries [32-38]. The inter-observer variability between 6 different coma scales used in children was assessed and the Paediatric Coma Scale had the highest inter-observer agreement [12]. In an attempt to standardise the way coma is measured in children, the British Paediatric Neurology Association (BPNA) has recommended the James adaptation of the GCS (Table 1.1b) as it takes into account the developmental immaturity in

small children, uses the same number of points as the standard GCS irrespective of the child's age, and is simple to use.

The determination of the GCS may be complicated by endotracheal intubation, sedation, and pharmacological paralysis [39]. In adults, the full GCS was not testable in up to 44% of the patients at the time of admission to a neurosurgery service [39]. Serious implications on the treatment required and prognosis may arise if the initial GCS is not scored accurately. In an attempt to overcome this problem, members of the Traumatic Coma Data Bank have proposed to assign a GCS verbal score of 1.1 to all adult head injured patients who have been intubated prior to the first GCS assessment [39]. However, this practice may significantly overestimate the injury severity.

Table 1.1a: Glasgow Coma Scale (GCS)

Scale	Criterion	Score
Glasgow Coma Scale (GCS)	Eye Opening	
	Spontaneous	4
	To speech	3
	To pain	2
	None	1
	Best Verbal Response	
	Orientated	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	None	1
	Best Motor Response	
	Obedying command	6
	Localises to pain	5
	Withdraws to pain	4
	Abnormal flexion	3
	Extensor response	2
	None	1

Table 1.1b: Modified GCS for Children

Scale	Criterion		Score
James' Adaptation of Glasgow Coma Scale	Eye Opening		
	Child > 5 yrs	Child < 5yrs	
	Spontaneous	As older child	4
	To speech	As older child	3
	To pain	As older child	2
	None	As older child	1
	Best Verbal Response		
	Child > 5 yrs	Child < 5 yrs	
	Orientated	Alert, babbles, coos, words or sentences to usual ability	5
	Confused	Less than usual or spontaneous irritable cry	4
	Inappropriate words	Cries to pain	3
	Incomprehensible sounds	Moans to pain	2
	None	None	1
	Best Motor Response		
	Child > 5 yrs	Child < 5 yrs	
	Obeys command	As older child	6
	Localises to pain	As older child	5
	Withdraws from pain	As older child	4
	Abnormal flexion	As older child	3
	Extensor response	As older child	2
	None	As older child	1

Table 1.2:

Scale	Criterion		Score
Sharples' Adaptation of the James' Adaptation of Glasgow Coma Scale	Eye Opening		
	Child > 5 yrs	Child < 5yrs	
	Spontaneous	As older child	4
	To speech	As older child	3
	To pain	As older child	2
	None	As older child	1
	Best Verbal Response		
	Child > 5 yrs	Child < 5 yrs	
	Orientated	Alert, babbles, coos, words or sentences to usual ability	5
	Confused	Less than usual or spontaneous irritable cry	4
	Inappropriate words	Cries to pain	3
	Incomprehensible sounds	Moans to pain	2
	None	None	1
	Intubated	Intubated	VT
Grimace			
Spontaneous normal facial/oromotor activity e.g. sucks tube, cough			5
Less than usual spontaneous ability or only response to touch			4
Vigorous grimace to pain			3
Mild grimace or some change in facial expression to pain			2
No response to pain			1
Best Motor Response			
Child > 5 yrs	Child < 5 yrs		
Obeys command	As older child		6
Localises to pain	As older child		5
Withdraws from pain	As older child		4
Abnormal flexion	As older child		3
Extensor response	As older child		2
None	As older child		1

A recent report from Britain proposed the use of a grimace score instead of the verbal component of the GCS while assessing the conscious levels in intubated children. (Table 1.2) [40]. This modified paediatric coma scale for intensive care practice has originated from the Sharples' adaptation of the James' modification of the GCS and was formulated on the basis that (i) non-verbal communication and verbal language are not totally independent, and (ii) both facial expression and grimace form an important part of non-verbal communication [40]. In a cohort of 73 critically ill children, the inter-observer reliability of this scale was found to be between moderate to good for all components assessed using the grimace score, and this was shown to be better than the verbal score in the whole group suggesting that the grimace score may be more useful in intubated patients [40]. However, this component requires further evaluations before formal introduction into clinical practice can take place.

1.2.5.2) Other System Injury Scores

Various other scoring systems, such as the Injury Severity Score (ISS), Revised Trauma Score (RTS), and Trauma Score-Injury Severity Score (TRISS), have also been used to classify the severity of head injury and are summarised below. However, these scoring systems often have very strict and detailed definition criteria or involve complex calculations limiting their applicability and usage in clinical practice particularly in the emergency room and the intensive care settings where prompt management decisions are often required. Thus, most of these scoring systems have been restricted to research practice.

1.2.5.2.1) Injury Severity Score (ISS)

This is an anatomical scoring system which gives an overall score for multiple injuries. For each injury present, an abbreviated injury scale (AIS) score is allocated to one of six body regions. The three highest AIS scores from the three most severely injured body regions scores are squared and added to produce an overall ISS. The range is between 0 and 75. The ISS correlates with mortality, morbidity, hospital stay and other measures of severity [41].

1.2.5.2.2) Revised Trauma Score (RTS)

This is a physiological scoring system and predicts mortality. It is based on the first set of examinations of the Glasgow Coma Scale (gcs), the systolic blood pressure (sbp), and respiratory rate (rr):

$$\text{RTS} = 0.9368 \text{ gcs} + 0.7326 \text{ sbp} + 0.2908 \text{ rr}.$$

The range is from 0 to 7.8408. The RTS correlates well with the probability of survival.

1.2.5.2.3) Trauma Score – Injury Severity Score (TRISS)

This determines the probability of survival using the ISS and RTS. Although the TRISS indicates the probability of survival for blunt or penetrating injuries, these are not differentiated in children [42].

1.2.5.2.4) The Paediatric Trauma Score (PTS)

The Paediatric Trauma Score (PTS) summates scores of weight, airway maintenance, systolic blood pressure, pupil responses, and the presence of an open wound or skeletal trauma. It indicates only a significant mortality risk and ranges from +12 to –6. A score of ≤ 8 indicates a significant mortality risk [43].

1.2.6) Outcome

1.2.6.1) Global Outcome Assessment

The Glasgow Outcome Scale (GOS) score has become the most widely used method to describe the global outcome after head injury in adults since it was first published in 1974. Although it is quick to administer and has clinically relevant categories, it does not take into account the special developmental considerations in the assessment of outcome in children with traumatic brain injury. Adelson and co-workers have modified the GOS for use in infants and children (Table 1.3) [44] but the modified GOS still does not consider behavioural morbidity which is common

after childhood brain trauma. Crouchman and colleagues further modified the five categories of the modified GOS to produce the eight points King's Outcome Scale for Childhood Head Injury (KOSCHI) analogous to the expanded adult GOS (Table 1.4) [45]. The scale places a high emphasis on concentration, behaviour and disinhibition which are common problems in children. It is intended to allow completion by either direct observation or from routine follow-up medical records, prospectively or retrospectively. The KOSCHI scale shows comparability with the GOS allowing comparison with adults as well as facilitating the transition of follow-up into adulthood.

Table 1.3: Modified Glasgow Outcome Scale

Category		Description
1	Dead	Dead
2	Vegetative	No interaction or cognition of outside stimuli.
3	Severely disabled	Significant gross motor or cognitive dysfunction requiring ongoing therapy, special schooling, or early intervention; minimal gaining of age-appropriate milestones.
4	Moderately disabled	Notable motor or cognitive dysfunction requiring ongoing therapy, special education, or early intervention; occasionally able to independently perform age-appropriate activities; gaining milestones though delayed.
5	Good Outcome	Good outcome with minimal dysfunction, return to school, independent age-appropriate activities, or continuing to achieve age-appropriate milestones.

Table 1.4: King's Outcome Scale for Childhood Head Injury (KOSCHI)

Category		Definition
1	Death	Death
2	Vegetative	The child is breathing spontaneously and may have sleep/wake cycles. He may have non-purposeful or reflex movements of limbs or eyes. There is no evidence of ability to communicate verbally or to respond to commands.
3	Severe disability	<p>(a) The child is at least intermittently able to move part of the body/eyes to command or make purposeful spontaneous movements; for example, confused child pulling at nasogastric tube, lashing out at carers, rolling over in bed. May be fully conscious and able to communicate but not yet able to carry out any self care activities such as feeding.</p> <p>(b) Implies a continuing high level of dependency, but the child can assist in daily activities; for example, can feed self or walk with assistance or help to place items of clothing. Such a child is fully conscious but may still have a degree of post-traumatic amnesia.</p>
4	Moderate disability	<p>(a) The child is mostly independent but needs a degree of supervision/actual help for physical or behavioural problems. Such a child has overt problems; for example, 12 year old with moderate hemiplegia and dyspraxia insecure on stairs or needing help with dressing.</p> <p>(b) The child is age-appropriately independent but has residual problems with learning/behaviour or neurological sequelae affecting function. He probably should have special needs assistance but his special needs may not have been recognised/met. Children with symptoms of post-traumatic stress are likely to fall into this category.</p>
5	Good recovery	<p>(a) This should only be assigned if the head injury has resulted in a new condition which does not interfere with the child's well being and/or functioning; for example:</p> <ul style="list-style-type: none"> • Minor headaches not interfering with social or school functioning • Abnormalities on brain scan without any detectable new problem • Prophylactic anticonvulsants in the absence of clinical seizures • Unsightly scarring of face/head likely to need cosmetic surgery at some stage • Mild neurological asymmetry but no evidence of affect on function of limb. Includes isolated change in hand dominance in young child. <p>(b) Implies that the information available is that the child has made a complete recovery with no detectable sequelae from the head injury.</p>

1.2.6.2) Mortality

TBI is the commonest cause of trauma death in children aged 1-15 years in England and Wales [46]. The mean mortality rate from childhood head injury calculated from 18 published studies was 5.1 ± 2.39 per 100,000 children per year [1, 3, 8-10, 12, 14-17, 20, 21, 25, 26, 46-49]. While the incidence of severe traumatic injury (GCS 3 - 8) has been decreasing it remains at 7-10% of all head injuries, and the mortality for this severely injured group is high at 20-40%. Although accidental injury mortality rates have fallen in the United Kingdom, certain population groups, such as children residing in less affluent areas, remain at greater relative risk [8, 50]. In Scotland, pedestrian accidents were the leading cause of brain trauma deaths in children [8, 9].

The incidence of severe childhood brain trauma mortality has declined steadily and significantly over the past two decades. The reason for this decline is likely to be multifactorial because of the simultaneous injury prevention efforts from the governments, parents, health care professionals, engineers and manufacturers of cars or playgrounds to improve safety and to reduce fatality among childhood accidents.

1.2.6.3) Morbidity

Approximately 3000 children every year acquire new neurological or cognitive disability as a result of head injury in the UK [51] and in Scotland, there are more than 100 new cases of disability occurring each year as a result of brain trauma for every 100,000 population [4].

Survivors of childhood brain trauma may suffer from a variety of deficits ranging from physical disabilities to behavioural and cognitive difficulties. Even mild head injury may lead to persistent cognitive and behavioural deficits [52, 53]. Morbidity experienced by different cohorts of childhood head injury survivors is difficult to compare because outcome assessments in children post brain injury require special developmental considerations. Neurodevelopmental processes continue, although often modified, after injury, measures of outcome must, therefore, relate to age appropriate expectations. Physical, behavioural, and cognitive aspects contribute varying degrees towards the overall morbidity depending upon the age at injury, interval since injury, injury severity, and injury independent factors.

One long term follow up study of childhood head injury indicated that 1/3 of the patients reported sequelae from the head injury with 1/3 of the complaints related to physical disabilities, another 1/3 were intellectual deficits, and the final 1/3 were emotional complaints [54]. A higher proportion of morbidity was reported in another study where 55% of patients reported symptoms and physical limitations were detected among 45% of these patients [55]. The most common neurological complaint in all reported series was headache, while musculoskeletal symptoms were the second most frequently documented symptoms. Learning difficulties occurred frequently after head injury and were experienced by 22 to 78% of children [54, 55].

1.3) DETERMINANTS OF OUTCOME AFTER BRAIN TRAUMA

1.3.1) Introducing Primary Brain Injury and Secondary Brain Insults

Brain trauma outcome is determined by the amount of brain injuries sustained during the moment of trauma and its subsequent clinical course. When trauma occurs, the initial mechanical forces applied to the head will cause injuries to the neurons, glial cells, and surrounding blood vessels. These are primary brain injuries and may, therefore, include epidural and subdural haematomas; subarachnoid, intracerebral, and intraventricular haemorrhages; traumatic axonal injuries; as well as brain contusions and lacerations. Additional brain injury occurs as a result of the pathophysiological response to the primary brain injuries, and these are called secondary brain insults which will also modulate neurological outcome.

The concept of secondary brain insults was first introduced in the late 1970s when one third of the brain trauma patients who died after admission to neurosurgical units were reported to have died at some point after the initial trauma [31, 56, 57]. The primary brain injury in these patients was judged to be not severe enough to cause death, but subsequent events resulted in fatal or disabling cerebral injury [31, 56, 57]. At the same time, evidence from post-mortem studies showed that over 80% of adults with fatal brain trauma had ischaemic brain damage despite the introduction of intensive care treatment regimes [58]. Secondary brain damage has also been shown to occur in children following brain trauma. Sharples' group reported the presence of 'avoidable' secondary brain insults in children with head injury [59], and hypoxic

brain damage has been shown to occur frequently in fatal accidental and non-accidental brain injury in infants and children [60-62].

Accident preventative measures have dramatically reduced the number of fatal childhood brain trauma but accidents continue to occur and little can be done to alter the extent of primary brain injuries once sustained. For patients who survive the initial brain trauma to reach hospital and subsequent neuro-intensive care, outcome is, therefore, related to the amount of secondary brain insults experienced [31, 56, 57, 59, 60].

1.3.2) Secondary Brain Insults

1.3.2.1) Secondary Physiological Derangements

In response to the initial brain trauma, serious systemic and intracranial physiological complications occur which include hypoxia, hypotension, raised intracranial pressure (ICP), reduced cerebral perfusion pressure (CPP), pyrexia and disturbances of global brain oxygen extraction ($SjvO_2$) [63]. These secondary physiological derangements may arise at any stage following the initial trauma and cause further brain damage through secondary brain injuring mechanisms. Deranged physiology is easily detectable clinically and prompt treatment may, therefore, reduce secondary brain insult and alter the outcome of brain trauma patients.

1.3.2.1.1) Frequency of Secondary Physiological Derangement after Brain Trauma

Prior to implementation of advance trauma care and active resuscitation at the scene of accidents, hypoxemia occurred in up to 30% of brain injured patients and arterial hypotension was found in 15% of patients on arrival to the Accident & Emergency Department [64-69]. Deranged physiology may also occur later in the clinical course of brain trauma in the Intensive Care Unit and during transfers. Miller and colleagues reported a high incidence (53%) of raised ICP (levels > 20 mmHg) among brain injured adults requiring intensive care in the late 1970s and early 1980s [65, 70] but data from the Traumatic Coma Data Bank (TCDB) indicated subsequently that an even higher proportion (72%) of intensive care patients suffered from intracranial hypertension following head injury [71].

In a prospective study involving 124 critically ill head injured adults, Jones and her colleagues examined up to 14 different physiological variables (such as ICP, arterial blood pressure, CPP, heart rate, oxygen saturation, core and peripheral temperature) recorded in minute resolutions during the patients' intensive care management [72]. They found that the majority (91%) of the brain trauma patients had deranged physiology regardless of the initial brain trauma severity, their age, or the admission Injury Severity Score (ISS) [72]. Andrews and co-workers demonstrated that 50% of brain trauma patients developed deranged physiology during intra-hospital transfers (mostly between the ICU and CT scanning suites) [73]. In addition, more than 65% also had physiological derangements in the ICU during the subsequent 4 hours of

continuous monitoring following the transfer [73]. These studies have highlighted that intracranial and systemic physiological complications occur frequently after brain trauma and the importance to continuously monitor and normalise brain trauma patients' physiology throughout their early clinical course.

1.3.2.1.2) Secondary Physiological Derangement and Brain Trauma Outcome

Brain trauma mortality and morbidity were increased significantly in patients who suffered from early hypoxia or hypotension following the initial trauma [63]. Similarly, the duration of hypotension, pyrexia, and hypoxemia that occurred in the intensive care were the most significant predictors of mortality [72]. One possible explanation is that deranged physiology affect oxygen and substrates (particularly glucose) delivery to the brain. When the physiological response to brain trauma becomes so severely impaired that brain perfusion and oxygenation is no longer sufficient to meet its metabolic need i.e. below the critical ischaemic thresholds, brain ischaemia occurs and causes further damage to the already injured brain, thereby worsening the prognosis.

1.3.2.2) Critical Care Neuro-trauma Management

Modern critical care brain trauma management aims to normalise cerebral and systemic homeostasis to prevent additional brain insults [74, 75]. This concept was first introduced by the late Professor JD Miller in Edinburgh during the 1970s.

Standardised treatment guidelines were developed locally to ensure rapid diagnosis and treatment of intracranial mass lesions, prevention of hypoxia, hypercapnea, fluid and electrolytes imbalance, anaemia, and arterial hypotension [76] among brain trauma patients. Its effectiveness in reducing head injury related mortality by as much as 20% quickly became apparent [76] with worldwide adoption of the practice which evolved into the basis of modern adult and paediatric neuro-trauma care.

The original critical care brain trauma management emphasized on the treatment of raised ICP [76], because intracranial hypertension was associated with poor outcome after brain trauma and was responsible for up to half of the brain trauma fatalities [70] and ischaemic brain damage [77-79]. Vigilant clinical examination and prompt access to neuro-imaging allow early identification and evacuation of intracranial haematomas [74, 76]. Introduction of neuro-intensive care and advancement in physiological monitoring technology enable prevention, early detection and treatment of cerebral oedema and intracranial hypertension with controlled mechanical ventilation, maintenance of adequate sedation and the use of muscle relaxants, osmotic diuresis, treatment of seizures and pyrexia, and pharmacologically induced coma with barbiturate [74, 76].

Despite vigorous treatment of intracranial hypertension, a significant proportion of fatal brain trauma patients in the 1980s continued to demonstrate the presence of ischaemic brain damage [58, 79]. At the same time, poorer brain trauma outcome was increasingly linked with longer duration of low cerebral perfusion pressure [63, 72]. The emphasis of brain trauma management was, therefore, shifted towards

maintenance of an adequate CPP while preventing and minimising other physiological derangements including raised ICP and hypoxia [74].

Although intensivists agree that it is important to normalise cerebral and systemic physiology after brain trauma, alarming differences have been reported in the intensive care management of brain injured patients in Europe and the USA during the 1990s [80-82]. This finding highlighted the need to develop standardised management guidelines for brain trauma. The Brain Trauma Foundation [74], European Brain Injury Consortium [83], and Scottish Intercollegiate Guidelines Network [84] have independently published intensive care management guidelines for adult traumatic brain injury with a general aim to normalise cerebral homeostasis. Treatment thresholds for ICP and CPP have been proposed in these guidelines [74, 83] which have become widely accepted in clinical practice across the world even though they have not been validated. Lack of threshold validation may be due to limitations in current methodology to determine and summarise abnormal physiology which only allows one dimensional derangement assessment at any one time e.g. the duration or magnitude of physiological derangement.

Much of the current literature on prevention, detection, and correction of secondary physiological derangements after brain trauma comprised of adult brain trauma studies. Very little research investigates the clinical aspects and pathophysiology in children with brain trauma apart from a few specialised studies [85, 86] which primarily examined cerebral blood flow, cerebral hyperaemia, CMRO₂, and A_jDvO₂. Brain trauma treatment principles from adult practice have been projected

and applied to manage infants and children after brain trauma without considering the age-maturation effect on intracranial haemodynamics. Because of this lack of sufficient data available in the paediatric head injury literature, considerable variations exist among different paediatric intensive care units across the UK and worldwide [6].

1.3.2.2.1) Lack of Normative Age-Related Intracranial Physiological Data

Normal systemic and intracranial physiology changes with age from birth through childhood and adolescence to the final adult values and may partly explain the difficulties in conducting research on this vitally important topic. Previous population studies have acquired normal acceptable ranges for heart rate and blood pressure among children of different ages [87] but normative data for ICP in children are more difficult to establish because of the ethical difficulties to measure it accurately through invasive means in healthy children. As a result, no information is available on the normal age-specific childhood cerebral perfusion pressure (CPP) values, which is calculated as the difference between mean arterial blood pressure (MAP) and ICP. This vital lack of knowledge on normative data has hindered clinical research progress and development of age appropriate brain trauma treatment guidelines. This difficulty was highlighted in the Pediatric Head Injury Guideline [75] endorsed by the following medical associations and societies: American Association for the Surgery of Trauma, the Child Neurology Society, the International Trauma Anesthesia and Critical Care Society, Society of Critical Care

Medicine, and the World Federation of Pediatric Intensive and Critical Care Societies.

Because of the significant gaps in normative age-related intracranial physiological data, almost all previous childhood TBI studies have grouped children of all ages together and applied a unified adult derangement definition to identify ‘abnormal’ intracranial physiology [88-91]. To use a standard definition of abnormal physiology for all ages without taking into account of the natural age-related physiological changes would be too simplistic and the transferability and usefulness of these results to clinical settings are questionable. A pilot study was, therefore, carried out by the Edinburgh Paediatric Traumatic Brain Injury Study Group to demonstrate the important associations between age-specific CPP derangement and outcome using pre-defined theoretical age-specific thresholds [92]. This study introduced a novel way to compare abnormal physiology among children of different ages [92]. However, like other studies of secondary physiological derangements, this study only assessed the relationships between the durations of the mean CPP values and neurological recovery without considering the effects of the derangement severity or the total burden of derangement on outcome [92].

1.3.2.2.2) Limitation in Research Methodology to Quantify Physiological Derangements

A measure that takes into account both the severity and duration of deranged physiology should provide a better reflection of the total burden of the potential

insult and allow better prediction of the outcome. Furthermore, an accurate measurement of the total quantity of derangements would allow testing of the theoretical age-specific thresholds to define the critical thresholds that differentiate normal and abnormal physiologies throughout different ages. Once these critical insult thresholds are identified, the amount of deranged physiology may then be refined as insults and validation of current therapies and other future novel treatment will then become possible.

1.3.2.3) Cellular and Molecular Pathophysiological Response to Brain Trauma

In addition to causing abnormal physiology, brain trauma also initiates a complex cascade of pathophysiological processes at cellular and molecular levels which include hypoxic ischaemic injury, oxidative stress response, excitotoxicity, neuro-inflammation, gliosis, and apoptosis. The current understanding of the precise roles and interactions of these pathophysiological processes is very limited but it is clear that some of these processes aid the repair of the injured brain while others cause further insults i.e. secondary brain injuring mechanisms. When trauma induced deranged physiology becomes insufficient to sustain cerebral metabolism, they become insults to the already injured brain and will also trigger these cellular and molecular pathophysiological brain injuring mechanisms.

1.3.2.3.1) Secondary Brain Injuring Mechanisms

1.3.2.3.1.1) Hypoxic / Ischaemic Injury

After brain trauma, hypoxic ischaemic damage occurs when the brain's oxygen and nutrient supplies become inadequate to meet its metabolic demands and may be the result of (i) primary brain injury when apnoea occurs following medullary injury [93], or (ii) secondary deranged physiological response to the initial brain trauma such as hypotensive shock, raised ICP, decreased cerebral perfusion pressure, and decompensated prolonged seizures. In cases of non-accidental head injuries, hypoxic ischaemic brain injury may rarely additionally be the result of suffocation which may have occurred to quell the infant's crying. The "big black brain" indicates particularly severe hypoxic ischaemic damage which is followed by death or the rapid development of cerebral atrophy. Hypoxic ischaemia is almost always an agonal event in most infant and childhood deaths as demonstrated by post-mortem evidence of severe hypoxic ischaemic brain damage found in 80% of fatal childhood accidental head injuries [60] and 77% of fatal non-accidental head injuries [61, 62].

Adequate perfusion is required to provide energy substrates and oxygen to meet the metabolic demands of the brain and to remove the metabolic waste products to prevent biochemical toxicity. Brain infarction, therefore, occurs following impaired perfusion which may be the result of hypotension, raised intracranial pressure, brain shifts with associated vascular occlusions, vascular spasm/obstructions, seizure or other causes of increased cerebral metabolism.

Hypotension does not result in uniform infarction of the whole brain [94] as ischaemia may occur in any major vessel from low perfusion pressure. The unique design of the Circle of Willis with its four feeding arteries means that there are areas within the brain where the pressures are equal, resulting in “no flow”. When the cerebral blood flow is severely impaired, these watershed zones between adjacent vascular territories are most susceptible to ischaemic damage because the cerebral arterioles are ‘end arteries’ without anastomosis. In the newborn, this is between centrifugal and centripetal arteries causing periventricular leucomalacia. In the older child it is between the middle and posterior cerebral arteries, causing infarcts in the pericentral white matter of the optic radiations and posterior temporal lobe, or between the anterior and middle cerebral arteries causing a wedge shaped infarct in the ‘leg area’.

Cerebral infarction as a result of brain herniations such as pericallosal or subfalcine shifts may follow specific patterns due to compression of the specific vital cerebral arteries. For examples, tentorial herniation may be associated with infarct in the areas supplied by the posterior cerebral artery and coning into the Foramen Magnum can cause compression of the posterior inferior cerebellar artery (PICA).

The main energy source for the brain is glucose which would produce 38 molecules of adenosine triphosphate (ATP) under aerobic conditions but only 2 under anaerobic conditions. Under anaerobic conditions the supply of glucose and removal of lactic acid becomes more essential since ATP is required for essential processes such as

neuro-transmitter formation, transport mechanisms, maintenance of membrane pumps and polarisation of membranes. Ischaemia is, therefore, far more dangerous than hypoxia because there is no supply of substrates (glucose or ketones) for ATP production, nor any way of removing lactic acid which results in the rapid development of neuronal necrosis.

Ischaemia initially causes swelling of the mitochondria within the neurons and astrocytes as a result of water retention. The neurons release potassium which is collected by the astrocytes and together with accumulation of glycogen within the astrocytes further swelling occurs [95]. Blood-brain barrier damage will result in leakage of proteins and blood, as in cases of haemorrhagic infarct, into the extracellular space through the endothelial cells. Finally, liposome rupture and neuronal cell death occur and coupled with the biochemical breakdown of tissue proteins result in (i) swelling of the infarct which further compresses capillaries, (ii) breakdown of blood-brain barrier, (iii) loss of cerebral auto-regulation, (iv) thromboplastin release causing sludging, and (v) liquefaction of the tissues.

The necrotic tissue may then be absorbed by macrophages leaving a cyst, as in periventricular leucomalacia, or may cause an astrocytic reaction replacing the dead tissue with the formation of a glial scar and capillary proliferation i.e. reactive astrogliosis.

Acute hypoxic ischaemia activates cellular production of adenosine and ACTH in an attempt to slow down cerebral metabolism to minimise the degree of cerebral

metabolic perfusion mismatch. Thus, a potential therapeutic window may exist to allow reduction of brain damage after hypoxic ischaemia. Additionally, a biochemical cascade is initiated which includes excitotoxicity, oxidative stress, neuroinflammation and pathways leading to programmed neuronal cell death (apoptosis) which may also be the targets of other neuroprotective measures.

1.3.2.3.1.2) Excitotoxicity

The main excitatory amino acid in the brain is glutamate [96]. Under normal circumstances, glutamate stimulation of the post-synaptic ligand-gated ion channel receptors (NMDA, AMPA, and kainite) is only transient. Excitotoxicity occurs when this stimulation is prolonged.

Glutamate concentration in the interstitial tissue is normally lower than the blood or intracellular glutamate levels, and its concentration is regulated by trans-membrane up-take of glutamate using the energy dependent sodium (Na^+) ion pump. Damaged neurons release glutamate into the interstitial fluid where it will accumulate to toxic levels if the blood-brain barrier is disrupted. The glutamate transport is impaired during ischaemia causing further increase in the interstitial glutamate level. When all energy supply is exhausted, membrane pumps fail and cause further sodium and water influx into the neurons. This results in cell swelling and subsequent release of glutamate [97]. In addition, there is excessive calcium influx [98] which may trigger activation of protein C, calpain I, phospholipase, xantine oxidase, nitric oxide, and oxygen free radicals [99, 100]. All these processes may cause further brain insults.

1.3.2.3.1.3) Oxidative Stress

Reactive oxygen species and reactive nitrogen species are produced in small amounts during oxidative metabolism in the mitochondria. After brain trauma, their production is enhanced with excitotoxicity [101]. Metabolic acidosis from reduced perfusion and anaerobic metabolisms may also induce oxidative stress. This may be the result of iron release from transferrin and ferritin caused by the low pH. The released iron may then act as a catalyst for the production of oxygen free radicals [102]. Neuro-inflammation has also been demonstrated to cause oxygen free radical productions [103].

Oxygen free radicals cause lipid peroxidation and affect the integrity of cell membranes [104]. This may cause damage to the blood-brain barrier [105] resulting in worsening of cerebral oedema. Free radicals may also oxidise proteins and DNA [106-108] resulting in either apoptosis or necrosis.

1.3.2.3.1.4) Neuro-inflammation

Inflammation is the reaction of vascularized living tissue to any form of local injury with the ultimate aim of achieving healing and repair. Intracranial inflammation (neuro-inflammation) initiated by brain trauma has become the focus of many clinical research studies and animal brain trauma models over the past few decades but the understanding of this complex pathophysiological cascade remains limited.

Cerebral ischaemia induced cellular energy failure causes pathological membrane depolarization and leads to cellular release of excitatory amino acids (EAA) [97] such as glutamate and aspartate. In addition, massive influx of calcium and sodium ions into injured cells [98] which will cause activation of various enzymes such as proteases, phospholipases, inducible nitric oxide synthase and xanthine oxidase. As a result, the arachidonic acid cascade is initiated with the formation of nitrogen- and oxygen-derived free radicals [99, 100]. Lipid peroxidation follows further damaging cell membrane and ultimately results in neuronal cell death [104, 106-108].

In addition to neuronal cell death, damage to endothelial cells and astrocytes can cause a disturbance of the blood-brain barrier (BBB) integrity, consequently leading to passive leakage of serum proteins into the intracranial compartment. The post-traumatic intracranial production and release of pro-inflammatory cytokines such as tumour necrosis factor (TNF)- α and interleukin 1 β (IL-1 β) further contribute to BBB dysfunction [109]. These inflammatory mediators have been linked with BBB damage and intracranial inflammation in a variety of experimental models [110, 111]. Vasogenic cerebral oedema occurs as a result of the increased BBB permeability leading to increased ICP and decreased CPP, thus aggravating cerebral ischaemia and the concomitant pathophysiological events.

Blood derived leukocytes are attracted across the disrupted BBB into subarachnoid space by endothelial and leukocyte adhesion molecules, all have been demonstrated to be up-regulated during the acute post-brain injury period. Locally released

chemo-attractant mediators such as α and β chemokines, brain derived chemotactic factor, arachidonic acid derived leukotrienes and activated complement fragments all contribute towards leukocyte recruitment into the intracranial compartment. The recruited leukocytes are likely to contribute towards further brain damage by release of proteolytic enzymes and reactive oxygen intermediates since several experimental studies [111-114] have reported an increased accumulation of neutrophils in the brain and their association with increased secondary brain damage and adverse outcome.

Constitutive *complement expression* in the normal CNS is low because of the intact BBB separating the vascular and brain compartments [115]. Elevated serum and CSF complement levels have been demonstrated following brain trauma [116, 117]. This may be the result of 'acute phase response' following trauma with hepatocytes producing complement proteins which subsequently leak into the intracranial compartment through the dysfunctional BBB [118-120]. IL-6 is a potent stimulator of acute phase response and its increased production following brain trauma is well documented [118, 119]. Synthesis of complement proteins by resident cells such as astrocytes and microglia within the CNS is also likely to occur post trauma and increased intracranial production of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6, and IL-8) have been demonstrated in many clinical studies [109, 121-126] and these mediators are known to induce the biosynthesis of complement proteins by CNS resident cells. Activation of the complement system within the CNS [127-129] is likely to contribute further to the local inflammatory response and thereby influencing outcome after brain trauma.

Anaphylatoxins such as C3a and C5a are generated through activation of the complement system and are potent mediators of inflammation. Their actions include (i) increasing vascular permeability via induction of BBB damage, (ii) chemotaxis (via up-regulation of cellular adhesion molecules expressions), and (iii) activation of blood derived inflammatory cells [130-132]. This results in further local tissue damages via release of protease and free radicals [133, 134]. The intracerebral activations of the terminal complement pathways result in the production of C5b-9 (MAC) which may contribute towards host cell death by inducing cell lysis (necrosis) [135].

Recent evidence suggests that C5a may contribute towards astrogliosis and scar formation as well as neuronal apoptosis by binding to the C5aR expressed by glial cells and neurons [136]. It has also been suggested that the recruited leukocytes may also play a role in the induction of apoptosis or programmed cell death.

1.3.2.3.1.5) Apoptosis

Apoptosis or programmed cell death has been demonstrated to occur after brain trauma in animal models [137, 138] and has generated intense search for its occurrence in human after brain injuries. Ng and co-workers in Singapore examined brain tissue from head injured patients excised during emergency craniotomy for evacuation of cerebral contusions with mass effect and found that variable expression of apoptosis related genes p53, bax, and bcl-2 [139]. They also

demonstrated the expression of the anti-apoptotic gene (bcl-2 gene) was associated with a positive correlation with survival [139]. In another study using the in situ nick translation (ISNT) technique, apoptotic neuronal cells had been detected in cortical contusion within 45 minutes of sustaining a blunt head injury with a peak incidence occurring at around 24 hours post injury [140]. This study also showed that the presence of apoptotic neuronal cells was detectable for up to 2 weeks after the initial injury [140].

Some authors suggested apoptosis inhibitors might be used to limit secondary neuronal cell death and may, therefore, potentially improve neurological recovery after various brain injuries. However, apoptosis is only one of the secondary brain injuring pathways and is in delicate balance with necrosis. Inhibition of one pathway may merely enhance the others.

1.3.3) Genetic Factors

Variation in brain trauma outcome despite introduction of neuro-intensive care suggests that other factors such as genetic makeup may influence outcome following brain injury. Over the past decade, the polymorphic Apolipoprotein E (APO E) gene has been suggested to affect outcome after brain trauma in adults because the possession of the APO E e4 allele has been associated with poorer outcome [141-143]. Furthermore, its gene product, apolipoprotein E (apoE) is involved in the lipid transport system within the central nervous system [144] which is thought to be particularly important to the repair process following neuronal injury. However, the

precise mechanisms through which APO E genotypes influence outcome after brain injury remain unknown.

Despite the independently growing brain trauma literature on secondary physiological derangement and genetic influence on outcome, no previous study in adults or children have investigated whether brain trauma patients in possession of the different APO E alleles suffer different burden of secondary deranged physiology. Clinical studies are urgently required to determine the relationship among APO E genotypes, secondary physiological insults, and outcome.

1.4) NEUROPATHOLGY IN FATAL TBI IN CHILDREN

Between 5 to 20% of acute childhood brain trauma requiring hospital admission have a fatal outcome [1, 145, 146]. Only a few large cohort studies of fatal TBI in children are available in the literature. Graham and colleagues [60] examined fatalities from accidental childhood brain trauma and found that the pathology distribution was similar to those seen in adults. They also found that hypoxic ischaemic brain damage was a frequent finding among their exclusive child cohort and its prevalence was higher than that previously reported in adult fatal brain trauma [60]. Neuropathology from non-accidental brain trauma differs significantly from accidental brain trauma. Geddes and co-worker examined the neuropathology of fatal non-accidental head injury and found statistically significant different patterns of age-related damages [61, 62]. They found that infants aged 2-3 months

have craniocervical axonal damage with global hypoxia, while children >1 year of age have severe extracranial injuries, and neuropathology similar to adults [61, 62].

Although brain trauma is the commonest cause of death in children, no studies have investigated whether implementation of modern trauma care has reduced the frequency of hypoxic ischaemic brain damage in childhood brain trauma.

1.5) OUTCOME PREDICTION DURING THE ACUTE PHASE POST BRAIN TRAUMA

Parents of critically ill brain trauma patients will want to know if their injured children will survive the injury, how well they will recover, and any long term disability to expect after recovery from the injury. They often ask clinicians to predict outcome soon after the occurrence of brain trauma. Accurate outcome prediction needs to be based upon accurate facts, which would require the patient to display pathognomonic features that only occur in a specific outcome. Irreversible brain stem damage is currently the only circumstance where clinicians can accurately and confidently advise the patient's family of a definite outcome i.e. death [147].

Difficulties to accurately predict brain trauma outcome during the acute phase post injury are expected because determinants of brain trauma outcome are multiple and heterogeneous. In addition, current understanding of the interaction between these brain injuring mechanisms remains very limited. Attempts have been made to use clinical features such as post-resuscitation GCS, duration of coma, length of ICU

stay, neuro-imaging findings, and physiological derangement thresholds to predict unfavourable outcome after brain trauma [148-159], but none of these measures offer prediction accurate enough for use in clinical practice.

Despite the vast quantity of literature available on the pressure signals of ICP, MAP and CPP, only a few describe their variability after traumatic brain injury. Early attempts to assess the variability using paper recordings were made [160-162] but proven difficult by the many technical difficulties which included lengthy traces, chart speed variations, and a lack of objectivity in the quantification and analysis of the data. Computerised physiological monitoring is the established norm in modern neuro-intensive care, but there have been few attempts to re-examine these early findings. It is possible that the variability of these pressure signals recorded over time after brain trauma in children may be important for outcome prediction.

Injured neuron and astroglial cells release brain specific proteins such as S100B protein, and neuron specific enolase which may be detected in the CSF and blood during acute phase of brain trauma [163-168]. In addition, cellular and molecular pathophysiological responses to brain trauma are modulated by biochemical mediators which are also measurable in the CSF and blood after brain trauma [122, 123, 125, 126, 169-176]. Clinicians have hoped to utilise these biomarkers to provide objective assessment of brain trauma severity and to predict outcome during the acute phase after brain trauma. As a result, increasing numbers of potential brain trauma biomarkers have been added to the literature describing their associations with injury severity or brain trauma outcome, but none have been translated into

clinically effective tools. This may be because most researchers have only investigated individual markers or small number of biomarkers of the same family at any one time. In addition, not all proposed biomarkers have been evenly investigated. Significant variations in sample type used, sampling time points, and data analytical methodologies exist between studies making comparisons difficult within the current literature. As a result, it is unknown whether some biomarkers may offer better injury quantification while others have more superior outcome predictive values.

1.6) SUMMARY

Brain trauma is the commonest cause of death and disability in children [46, 177]. Hypoxic ischaemic brain damage is a frequent finding in fatal childhood brain trauma [60-62] indicating that its prevention may improve traumatic brain injury outcome.

Attempts to translate promising novel neuro-protective strategies from the laboratory into clinically effective therapies have been disappointing. For those who survive the initial brain trauma to reach hospital, the current best treatment option is to prevent secondary cerebral ischaemia by normalising cerebral and systemic haemodynamics [74, 75]. Because bedside measurement of cerebral metabolisms is not possible, clinicians use cerebral perfusion pressure as a guide of the driving pressure for cerebral blood flow. Treatment thresholds for raised ICP and low CPP have been proposed and become widely accepted in adult brain trauma management

[74] despite the lack of threshold validation. Current methodology to quantify deranged physiology is limited to a single dimensional assessment (e.g. duration or magnitude) at any one time, and may be the reason why threshold validation has not been possible.

Management of childhood brain trauma is further complicated by the lack of normative data on the age-related changes in intracranial physiology. As a result, some paediatric intensivists apply the well accepted but non-validated adult ICP and CPP treatment thresholds to treat brain injured infants and children, while others anecdotally adjust the thresholds to levels they feel appropriate to the patient's age. The Edinburgh Paediatric Traumatic Brain Injury Study Group has hypothesized age-related minimum CPP levels [92] which require validation before they may be developed into clinically meaningful treatment thresholds.

Secondary brain injuring mechanisms at cellular and molecular levels are complex and it is unclear whether a particular pathway has higher modulating effect on brain trauma outcome than others. They are modulated by biochemical mediators which may be detectable in systemic circulation and may, therefore, be potential objective indicators of brain trauma severity and predictors of outcome. The number of potential brain trauma biomarker has increased over the past decade but none has been translated into clinically useful tools. Difference in research methodology including sample types, sampling time points, biomarker studied, and data analysis may explain the difficulty to develop brain trauma biomarker usage. Identifying the biomarkers that best predict unfavourable outcome may highlight more influential

secondary brain injuring pathway and re-direct future research focus which may then enable identification of potential novel treatment points.

Genetic polymorphisms may be a determinant of brain trauma outcome. Possession of the APO E e4 allele has been associated with poorer outcome after brain injury in adults [141-143] and its gene product is involved in the CNS lipid transport system [144] which is thought to be particularly important in neuronal repair. It is unknown whether brain trauma patients with the different APO E genotypes respond differently to deranged physiology after brain trauma.

1.7) HYPOTHESES & AIMS

We hypothesize that:

1. Accurate quantification of the total burden of CPP derangement (i.e. assessing both duration and magnitude simultaneously) after brain trauma has better correlations with unfavourable outcome than using a single dimensional assessment of deranged CPP, and allows critical age-related minimum (insult) CPP thresholds to be defined.
2. APO E genetic polymorphisms modulate brain trauma outcome by affecting the different allelic carriers' response to brain trauma and the amount of CPP insult they experienced with carriers of the APO E e4 allele experiencing the largest amount of CPP derangement.

3. Reduction of CPP insult during critical care management will reduce cerebral ischaemia and improve outcome after childhood brain trauma.
4. Acute serum inflammatory marker levels have more superior prognostic values for unfavourable outcome after childhood brain trauma than those of brain specific proteins.

In order to test the above hypothesis in this thesis, we aim to:

1. Develop a method that quantifies the total burden (i.e. duration and magnitude) of CPP derangement and to assess its relationship with outcome after childhood brain trauma.
2. Validate the hypothetical age-related minimum CPP thresholds and to define the critical age-related CPP thresholds.
3. Determine whether children with different APO E genotypes experience different burden of physiological derangements, and their relationships with outcome.
4. Assess the predictability of 8 different serum biomarkers levels for unfavourable outcome after brain trauma.
5. Determine whether using 2 biomarker levels have more superior outcome prediction than using individual biomarker level.

6. Determine the relationship between the pre-morbid burden of CPP derangement and the amount of hypoxic ischaemic brain damage in fatal brain trauma.

CHAPTER 2: CRITICAL AGE-RELATED CEREBRAL PERFUSION PRESSURE THRESHOLDS AND QUANTIFICATION OF THE TOTAL BURDEN OF CPP INSULT

2.1) INTRODUCTION

Normalising cerebral and systemic homeostasis after brain trauma improves outcome [63, 65] and remains the principle management aim of modern neuro-intensive care [74] since its introduction over three decades ago [76]. It is unclear from the literature whether intracranial hypertension or low cerebral perfusion pressure best predict outcome after brain trauma. For management of adult brain trauma, treatment thresholds for raised intracranial pressure (ICP) and low cerebral perfusion pressure (CPP) have been proposed and become widely accepted in clinical practice without formal validations [74, 84].

The intensive care management of childhood brain trauma is based upon the same principle as adult neuro-intensive care but there is no agreement on the optimal treatment thresholds for raised ICP and low CPP for children of different ages [75]. This is because of the insufficient knowledge on the normal intracranial physiological variations with age maturation as previously discussed in Chapter 1. Currently, many paediatric centres merely apply adult treatment thresholds without considering the age-maturation effect on the intracranial physiology, while others anecdotally adjust their treatment thresholds with ill defined age groups. There is, therefore, a pressing need to define critical thresholds that enable identification of

secondary ischaemic brain insults, and to develop age-related treatment thresholds for raised ICP and low CPP so that outcome of childhood brain trauma may be improved.

The importance to consider age-related intracranial dynamics and systemic physiology in childhood brain injury is highlighted over the past decade. Jackson and colleagues used three different CPP (70, 60, and 50 mmHg) and ICP (12, 14, and 24 mmHg) thresholds to determine CPP and ICP insults in ten head injured children aged less than 16 years and demonstrated that ICP increased significantly between the CPP < 60 and CPP < 50 mmHg analyses [90]. This pilot work did not correlate with outcome assessment and their chosen thresholds had no physiological basis. In another report, based upon a single point on a Receiver Operator Characteristic (ROC) curve that had been created using a minimum of a rolling mean CPP in 84 children aged 3 months to 16 years, Chambers suggested absolute levels of 45 mmHg in children and 55 mmHg in adults for clinical CPP management [91]. Although this report attempted to introduce a different threshold for childhood management, it failed to consider the age-maturation effect throughout infancy and childhood and suggested only one threshold for the entire childhood which would range from toddlers as young as 2 year olds to adolescence of 16 years of age who will have adult physiology [91].

Jones and co-workers from the Edinburgh Paediatric TBI Research Group demonstrated that the duration of age-specific abnormal CPP predicted unfavourable outcome ($p=0.004$) and mortality ($p=0.003$) [92]. They proposed using pre-defined

theoretical age-specific minimum CPP levels to define abnormal CPP in a series of critically ill brain injured children aged less than 15 years of age [92]. As discussed previously in Chapter 1, detailed age-specific normative data for blood pressure exist in the literature but there is only incomplete age-related information on ICP causing difficulties in defining the normative values of CPP throughout childhood. Minns had tabulated the normal values of ICP as described by earlier workers, and had calculated the mean upper normal limits for specific age groups by plotting a graph of best fit [178]. However, these theoretical normative values for ICP are still not sufficient for calculation of theoretical age-specific minimum CPP thresholds. Given that ICP should be low and contribute very little towards the normal CPP in healthy infants and children, the Edinburgh Paediatric TBI Study Group hypothesizes that the theoretical lowest acceptable age-specific thresholds for CPP should be the same values as the lowest acceptable MAP by age [92]. This is because assuming ICP is zero, the lowest possible driving pressure for cerebral blood flow that may perfuse the brain must be the same as the lowest acceptable MAP. This study was the first to demonstrate the significant predictive value of age-specific abnormal CPP as defined by these theoretical age-specific thresholds on outcome after paediatric head injury and also provided a novel approach to study childhood brain trauma [92]. However, the thresholds used were purely theoretical and would require validation.

Many methods exist in the current literature to quantify the amount of ICP and CPP derangement reflecting the lack of standardised method to assess pressure insult. However, all these preceding studies relating ICP / CPP to outcome in traumatic and non-traumatic encephalopathies have focussed on individual excursions of pressure

beyond the currently accepted thresholds in clinical practice or the duration of the derangements. These levels lack a secure evidence base. In addition, these approaches concentrate on a single summary measurement, for examples means over hourly recordings or other time-frames, maximum or minimum values, percentage duration of derangement, and do not combine both severity and duration of derangement. Furthermore, these are pragmatic and loose much of the detail by virtue of averaging the peaks and troughs of the pressure recording. Lack of suitable methods to accurately quantify the total burden of secondary physiological derangements has hindered any attempts to determine age-related thresholds, validate current treatment regimes, and to develop novel therapies. A measure incorporating both degree and duration would theoretically be a better reflection of the total potential insult and allow for determination of thresholds as well as validation of current treatment regimes, thereby injecting evidence base approach into critical care medicine.

2.2) HYPOTHESES & AIMS

We hypothesize that inadequate cerebral perfusion pressure affects outcome after childhood brain trauma more than raised intracranial pressure. In addition, we hypothesize that unfavourable outcome prediction after childhood brain trauma is better achieved with assessment of the total burden of CPP insult (i.e. both duration and magnitude) than any of the traditional single dimensional assessment of CPP insult. Finally, we hypothesize that our theoretical age-specific minimum CPP levels

are critical thresholds which define CPP insults and provide the best predictability for outcome.

The aims of this chapter are to test the above hypotheses and will be achieved through (i) determination of the predictability of outcome of the various routinely monitored physiological parameters in the intensive care settings, (ii) the development a novel index that summarises both time and depth of derangement for ICP and CPP using detailed physiological data in minute resolutions; (iii) determining the sensitivity and specificity of the index to outcome; and (iv) applying this index to determine the age-related critical CPP thresholds in relation to childhood traumatic brain injury.

2.3) PATIENTS AND METHODS

2.3.1) Design of the Study

A prospective observational study was conducted to determine the relationship between secondary physiological derangement and outcome after childhood brain trauma. Children aged less than 16 years who had sustained traumatic brain injury and required intensive care management were included in the study with the exception of those who had a previous history of brain trauma. Local ethical and hospital management committees approved the study protocol. Parental consents were obtained prior to inclusion in the study.

2.3.2) Patients

A total of 128 brain trauma children were enrolled in the study and they were recruited from the regional paediatric neuro-surgical and intensive care centres based in Edinburgh and Newcastle upon Tyne. Of the whole cohort, 52 patients originated from the pilot study [92], 34 patients were recruited from the Royal Hospital for Sick Children (RHSC) in Edinburgh, 36 were recruited from Newcastle General Hospital (NGH), and 6 were from Western General Hospital (WGH) in Edinburgh. The original pilot group fulfilled the same inclusion criteria as our current cohort. 81 children were aged between 2 and 16 years and had continuous ICP monitoring. Detailed analyses of ICP and CPP (including the development and validation of the novel Cumulative Pressure-Time Index) were, therefore, conducted on these 81 children.

Patients were classified according to the post-resuscitation summated GCS into severe brain trauma ($GCS \leq 8$ with no eye opening i.e. E1), moderate brain trauma ($GCS 9 - 12$), and mild brain trauma ($GCS 13 - 15$) associated with other significant injuries sufficient to give an ISS of at least 16. The ISS score was chosen to ensure that patients with epidural haematomas of less than 100 ml were included.

All the recruitment units including that of the pilot group were specialist paediatric and adolescence intensive care units and the patients were managed by experienced neurosurgeons, paediatric intensivists and specialist critical care nurses. Mechanical ventilation and ICP monitoring were employed (i) after evacuation of an intracranial

haematoma where the patients' GCS was ≤ 8 ; (ii) where there was a diffuse cerebral injury and GCS ≤ 6 ; (iii) where there was CT or operative evidence of brain swelling; and (iv) where other injuries dictated the need for ventilation. Invasive monitoring was employed as clinically indicated. Physiological data was, therefore, only collected for those parameters judged by clinical staff to be applicable. The management guidelines for all Units were similar including therapeutic goals for maintaining CPP ≥ 50 mmHg, and ICP ≤ 15 mmHg for children aged 0 – 13 years, and for those aged 14 – 15, CPP and ICP were maintained ≥ 60 mmHg and ≤ 20 mmHg respectively. These guidelines were set for all children and had been in use prior to the commencement of the study.

Physiological data was collected until clinical monitoring ceased, even when this was for several weeks, once the patient was enrolled into the study. Clinical notes and nursing charts were reviewed. Admission and clinical details were recorded prospectively which included data on the cause and nature of the injury, age, GCS on admission and after acute non-surgical resuscitation, pupil responses, the results of X-rays and neuro-imaging such CT and MRI scans, operative and treatment details, and inter-hospital transfer details.

2.3.3) Data Collections and Analyses

2.3.3.1) Physiological Data

2.3.3.1.1) Original Data Collection Plan

Mobile computer data collection (CDC) systems were to be connected to ICU bedside monitors with RS232 card pre-installed. We initially planned to use a data acquisition software called Edinburgh Monitor© to download and store the continuous real-time physiological data in minute resolutions. Although the Edinburgh Monitor© Software was designed to read physiological data from a wide variety of bedside monitors, it was unable to detect pressure signals from the stand-alone (i.e. non-networked) Hewitt Packard Merlin monitors that were used in the ICU at the Edinburgh RHSC. The only parameters downloadable from these monitors were heart rate, oxygen saturation and temperature recording.

The creator and programmer of Edinburgh Monitor© (Dr. Tim Howells) attempted to create a software patch to read the pressure signals from the Hewitt Package Merlin monitors but was not successful. Networking all the bedside monitors in the RHSC site to a central terminal was an alternative approach and would enable storage of physiological data in minute resolutions. The stored data could then be exported using the Hewitt Packard network software into a format compatible with the Edinburgh Browser© software for analyses. However, this option would incur

expenses in excess of £20000. With a limited financial resource for the project, this option was excluded as a realistic solution.

ICUPilot™ software, produced by CMA Microdialysis, had the ability to download and store all physiological signals including pressure signals from Hewitt Packard Merlin monitors. In addition, it was able to export the stored data in .csv format which was a computer file format known to be compatible with the Edinburgh Browser© programme. Dr. Magnus Hedberg, the Head of Development of CMA Microdialysis, kindly offered to provide a free complimentary licence for our Paediatric TBI Research Group to use the software throughout the duration of our study.

2.3.3.1.2) Actual Data Collection Methods

Detailed methods for data download, storage and exportation on the three recruitment sites are summarised below.

Two mobile CDC systems with ICUPilot™ software installed were available at the RHSC site for connection to the stand-alone bedside monitors to download and store the routine continuous real-time minute-by-minute physiological recordings. Four of the six bedside monitors had the required RS-232 cards installed and were correctly configured (band width 19200) to communicate with the ICUPilot™ software. This set up did potentially limit the number of patients that could be monitored spontaneously and had a theoretical risk for the need to exclude a suitable patient if

both of the CDC systems were in used. However, in reality no patient was excluded as a result of this arrangement. ICUPilot™ exported the stored data for off-line analyses using the Edinburgh Browser© programme.

The bedside monitors in the ICU at the Edinburgh Western General Hospital (WGH) and the PICU at Newcastle General Hospital (NGH) were networked to a central terminal for storage of the minute-by-minute physiological data and therefore, posed no restriction to the number of suitable patients that could be monitored spontaneously. The stored physiological data were then exported using the WardWatch© software in the Edinburgh WGH site and the Critical Care Physics© (Regional Medical Physics Department Reporting) Software in Newcastle for analyses using the Edinburgh Browser© programme.

2.3.3.1.2) Physiological Parameters Measured

Physiological parameters routinely monitored continuously in brain trauma children include:

- heart rate,
- respiratory rate,
- mean ICP (measured by fibre-optic intracranial pressure monitor),
- systolic, diastolic and mean arterial blood pressure (measured with a standard intra-arterial line with the pressure transducer zeroed at the level of the external auditory meatus),
- CPP (the calculated difference between MAP and ICP),

- oxygen saturation (measured by a pulse oximeter), and
- core and peripheral temperature.

In addition, interventions (e.g. drug therapy, ventilation changes, nursing procedures or other textual notations) and observations (GCS and pupil responses) were recorded and stored in real time.

2.3.3.2) Outcome Data Collection

Global outcome of the survivors was assessed at 6 months post injury using a structured parents / carer questionnaire and was categorized using the Modified Glasgow Outcome Score (GOS) and King's Outcome Score for Children with Head Injury (KOSCHI). Fatal cases were assigned GOS or KOSCHI 1 for analyses. Two independent investigators blinded to the physiological and demographic data conducted outcome scoring for all patients.

Patients were dichotomised into the following groups for analyses:

- (1) Alive vs Mortality (i.e. dead)
- (2) Independent (GOS 4 and 5) vs Poor outcome (GOS 1 –3)
- (3) Best (GOS 5) vs Rest (GOS 1 – 4)

2.3.3.3) Data Analyses

2.3.3.3.1) Choosing Age-Specific Thresholds (Age Index)

The interpretation of the recorded data relied upon comparisons with accepted normal ranges. Age-specific threshold for each physiological parameter was, therefore, defined prior to data validation and analyses.

A literature review was undertaken to identify previously published data describing the normal values by age for each of the specific physiological parameters of interest namely heart rate, arterial blood pressure (systolic and mean), ICP, CPP, oxygen saturation, and core temperature [92]. A table of thresholds for physiological derangements for children by age was then constructed (Appendix I).

2.3.3.3.1.1) Heart Rate

Previously published normal ranges of heart rate in neonates and children of different ages have showed that after an initial rise in heart rate between birth and about 3 months, resting heart rate slows as the child grows. These values have been adopted as the pre-defined age-specific heart rate thresholds for this study and no gender difference has been applied to this study.

2.3.3.3.1.2) Hypoxia

Hypoxia is defined as having an arterial PaO₂ of 60 mmHg or less which is the equivalent of having an oxygen saturation of less than or equal to 90% as measured by pulse oximeter [179]. For the purpose of this study, hypoxia threshold for all age group was defined as having an oxygen saturation of 90% or less.

2.3.3.3.1.3) Pyrexia

The normal resting core temperature quoted for adult and children ranges from 36.3⁰C to 37.7⁰C [180]. The threshold for pyrexia in this study was taken as 38⁰C.

2.3.3.3.1.4) Arterial Blood Pressure

Normative data on arterial blood pressure in children had been acquired in several major studies [87, 181-183]. The largest and most comprehensive data originated from the US Second Task Force of 1987 which measured blood pressure in over 72 000 children aged between newborn and 20 years old in the sitting position using a standard clinical sphygmomanometer [87]. The published results included age and gender specific data for systolic and diastolic blood pressure. MAP was not given in either of the Task Force publications, and those described by Shann's group in Australia, although commonly used, were not year-specific. MAP, was, therefore, calculated from the Task Force values, having first identified the ranges for diastolic BP (mean \pm 2 SD). Mean pressure is usually given as the diastolic pressure (D) plus

one-third of the pulse pressure, where pulse pressure is the difference between the systolic (S) and diastolic (D) pressures: thus, $MAP = [D + (S - D) / 3]$.

Because there is no comparable data available for UK children, we have adopted values from this American Study as our blood pressure thresholds. Data were summarised for our study for each age group, and are detailed below: using the Task Force tables of boys' and girls' data, the normal ranges (calculated from the mean \pm 2SD) were found. Where there were differences between the genders, the lowest and highest were used to give the widest possibility range for this study since it was not our intention to consider sex and height differences. In practice, this made a potential difference of approximately 1 mmHg at each end of the systolic BP range, so only fractionally affected the mean blood pressure (MAP), which was rounded to the nearest integer.

2.3.3.3.1.5) Intracranial Pressure (ICP)

Accurate measurement of intracranial pressure requires invasive monitoring and given the ethical difficulties of measuring it in healthy normal subjects, normative data for ICP in infants and children is sparse. Much of the available information on childhood ICP has originated from the neurology literature i.e. measurements taken from infants and children who had already presented to the medical service with a variety of symptoms. These patients did not necessarily have a neurological condition, but their clinical presentations warranted investigations for raised ICP. Comparisons between studies were made difficult because these cohorts often

included infants and children of different ages i.e. some continued to have open fontanelles while others' cranial sutures had already fused which might influence the ICP recordings. Furthermore, ICP measurement sites and technique varied between studies further complicating comparisons between studies.

Despite these potential difficulties in comparing existing childhood ICP data in the literature, fairly similar ranges of ICP values have been quoted by these studies as being normal ranges for infants, children and adults. Moront's group considered normal ICP for children and adults as being less than 15 mmHg and a recording of less than 8 – 10 mmHg for infants [184]. Mann and Punt stated that ICP increases with age and reported a value of 8 mmHg to be normal in the older child [185] while Welch found normal CSF pressure in young children to be between 2 – 10 mmHg [186]. Lam recently gave adult normal ICP values as 8 – 18 mmHg, and for children between 2 and 4 mmHg without specifying the age but stressed the need for further evaluation [187].

Previously published normal ICP values were tabulated by Minns who had also calculated the mean upper normal limits for specific age groups [178]. Most authors agree that mean ICP in children rises with age, but remains less than adults. We used the figures given by Minns [178] as our thresholds, which were rounded to the nearest whole number, as required by our data analyses system. Plotting those values with a line of best fit provided the missing yearly increments.

2.3.3.3.1.6) Cerebral Perfusion Pressure (CPP)

Cerebral perfusion pressure (CPP) has been used as a way of assessing the intravascular pressure gradient across the brain. CPP is calculated as the difference between MAP and ICP. Under ideal circumstances, CPP can act as a useful guideline to ensure that systemic blood pressure is adequate to perfuse the brain in the face of increasing ICP.

No composite charts or tables of CPP were found to suggest normal lower limits in children of different ages, although some authors have given values of between 40 and 50 mmHg under normal conditions, without specifying age [184, 188-190]. A few notable studies of CPP have included children [90, 91, 191], but these have used the previously accepted adult value of 60 mmHg, or more recently 70 mmHg [192]. In adults, the lowest normal limit of CPP in brain trauma patients is usually empirically considered to be the same numeric value as the lowest acceptable limit for MAP [192-195]. For example, if 70 mmHg was the lowest limit of acceptable MAP, then 70 mmHg was also the lowest acceptable CPP in adults. While the range of normative data for blood pressure is quite precise (allowing calculation of the mean of MAP), the normative data on children's ICP is thin with only maximum values suggested by the literature for a few age groups. The values we have used have been deducted from references to normal children's ICP and are, therefore, hypothetical. We have used the lowest acceptable mean MAP value as the lowest acceptable CPP value, which is likely to be numerically as accurate as an estimation of the range of ICP. Accordingly, CPP was considered normal if > 45 mmHg in 1

year-olds increasing to > 58 mmHg at 15 years of age. It should be noted that to maintain a normal CPP, ICP and MAP need not both be in the normal range at the same time, i.e. CPP could still be normal if ICP rises above normal, but MAP is maintained at a sufficiently high level.

2.3.3.3.2) Physiological Data Validation and Analyses

Edinburgh Browser©, a specially developed minute-by-minute data analysing programme, was used to analyse the recorded data 'off-line' [72, 92, 159]. It was able to scrutinise and validate the data to exclude invalid recordings or artefacts such as probes detachment during patient care procedures, arterial line being flushed during blood sampling, calibration errors, or computer disconnections. These invalid data or artefacts were discarded. Any data recorded during the last 4 hours before death i.e. premorbid data were also excluded from the analysis if the patient underwent brain stem death tests.

Using predefined thresholds for each parameter by age, Edinburgh Browser© could then identify the validated physiological data which was out-with these preset norms i.e. abnormal physiology [72, 92]. Abnormal but valid physiology such as those associated with nursing care procedures or physiotherapy etc was retained. Physiological derangements were deemed to have occurred only when the abnormal recordings persisted for at least five consecutive minutes. This rule applied to all physiological parameters with the exception of temperature as it had a much slower rate of change. For a temperature derangement to be identified, the recording must

be outside the preset normal ranges for at least one hour. Where the derangement persisted beyond 5 minutes and judged to be 'real', the total derangement time recorded would include this initial 5 minutes. The derangement was deemed to have ceased when the recording returned to values within the preset normative values for 5 uninterrupted minutes or 1 hour in the case of pyrexia.

After validation and application of the age index for each child, Edinburgh Browser© was used to calculate (i) the overall start and stop dates and times of monitoring and the total number of minutes of monitoring time; and (ii) for each physiological variable, the duration (in minutes) of the invalid times, and the number of episodes and total duration of derangements. Total monitoring time was defined as the longest duration of any monitored modality. The cumulated deranged physiology time (DPT), both the absolute value and as a proportion of total monitoring time, was then calculated for each variable, for each child.

2.3.3.3.3) Logistic Regression Modelling & Discrimination Assessments - Misclassification Rate & Receiver Operator Characteristic (ROC) Curves

Since the clinical course of each patient could be different, the cases used for logistic regression analyses were restricted to those who had ICP monitoring available, and the data limited to those collected while an ICP monitor was in situ (i.e. the acute monitoring time). ICP monitoring was continued as long as required for clinical management of raised ICP. The likelihood of later derangements was then felt to be

reduced. This was referred to as the acute monitoring time, i.e. the ICP monitoring time excluding the pre-terminal 4 hours in those cases that underwent brain stem death testing. Only cumulated duration of derangements (absolute and percentage) were studied as there were insufficient patients in each year group to attempt to categorize the physiological data to reflect the magnitude of the derangement, or to use epochs since injury.

Logistic regression modelling was used to assess the prognostic significance of the demographic variables in a method similar to that described by Signorini and colleagues [159]. Each variable was initially fitted in a univariate model to assess the functional relation with outcome. For categorical variables, categories were merged as necessary to give fewer and simplified variables. Similarly, the duration and percentage duration of physiological derangements occurring during the acute monitoring time were assessed using a univariate model. The next step used multivariate modelling of the variables, using functional relations developed in the univariate models in a multivariate setting. The Hosmer & Lemeshow (HL) goodness of fit test statistic was used to assess calibration [196]. The discrimination was assessed by calculating both the misclassification rate and the area under the ROC curves for each model.

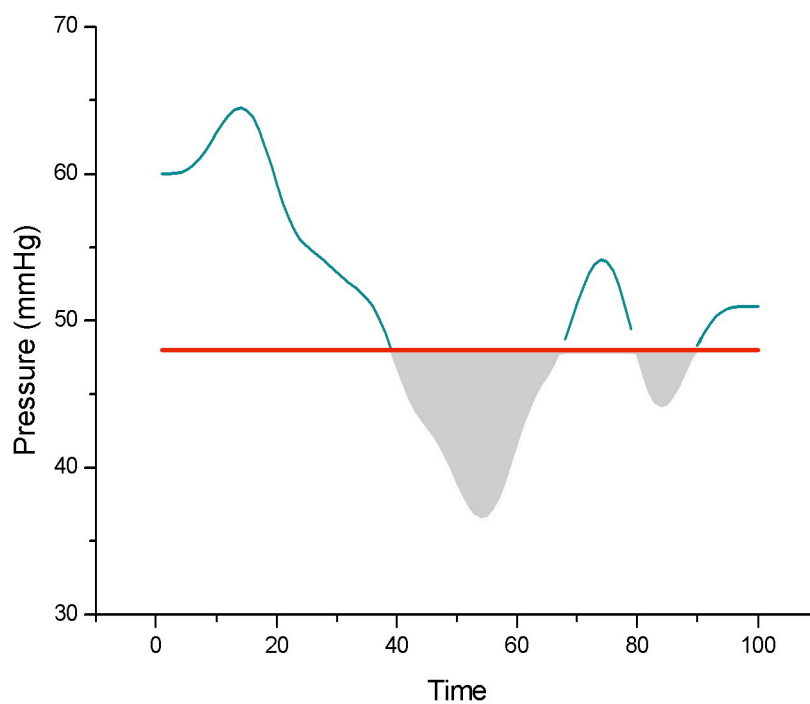
2.3.3.3.4) Derivation of the Cumulative Pressure-Time Index (PTI)

For each patient recording, the total area between the continuous threshold and the recorded CPP tracing was calculated. For the duration of each CPP insult detected

by the Edinburgh Browser© program the difference between the age threshold and the recorded CPP value was determined for each minute value. These were then summed to produce what we have termed the Cumulative Pressure-Time Index (PTI). Thus, PTIc is defined as the area below the pre-defined age-related threshold limits and the plot of CPP against time as shown in grey areas in Figure 2.1, and can be mathematically described by:

$$\Sigma(\text{CPP}_{\text{threshold}} - \text{CPP}) \times t_{\text{sample interval}}$$

Figure 2.1:



2.3.3.3.4.1) Calculation of the PTI for CPP (PTIc)

2.3.3.3.4.1.1) Allocation of Age Groups

As the normal ranges for physiological parameters such as blood pressure, intracranial pressure, and cerebral perfusion pressure vary with age from birth until about mid teenage years, it was necessary to factor this into the analysis. The values change rapidly during the first 12 months of life but insufficient cases with brain injuries in these early years were available, and to use annual increment would likewise have required more subjects. Our patients were divided into three groups using the following age bands: those aged 2 – 6 years, 7 – 10 years, and 11 – 16 years for the purpose of this analysis. Using our pre-set age-specific CPP thresholds, a theoretical mean CPP threshold was calculated for each age band and they are summarized as follows:

AGE	Mean CPP Threshold
2-6 years	$\leq 48\text{mmHg}$
7-10 years	$\leq 54\text{mmHg}$
11-15 years	$\leq 58\text{mmHg}$

PTIc was used as a test level of outcome prediction by making comparisons of the PTIc values between children with different recovery. Taking all the patients with independent outcome, a PTIc level could vary from 0 to its maximum value and the sensitivity and specificity of the index were calculated. The sensitivity of PTIc

would fall as the PTIc level was raised (fewer independent outcomes with greater insult severity), whilst the specificity would rise with an increasing level of PTIc (greater number of poor outcomes with increasing insult severity).

Using the values of specificity and sensitivity calculated at each threshold level, receiver operator characteristic (ROC) curves were plotted for independent outcome versus poor outcome, mortality (dead versus the rest) and morbidity (good recovery versus all other outcomes).

2.3.3.3.4.1.2) Determination of the Age Band Related Critical CPP Thresholds

In order to investigate the effect of different threshold levels for insult detection and whether these might better differentiate outcome, each age band threshold was reduced first by 10% and then by 20% and the PTIc recalculated. The age thresholds were then increased by 10% and the PTIc calculated again. Using the independent versus poor outcome dichotomy ROC curves were plotted for each of the new threshold test levels and the area under each curve determined.

2.3.3.3.4.2) Calculation of the PTI for ICP (PTIi)

Using the same methodology, a similar index was calculated for ICP by calculating the total area *above* the age threshold and the recorded ICP tracing. Again the effect

of altering the thresholds was investigated by reducing the thresholds by 10% and then increasing them by 10% and then 20%.

2.4) RESULTS

Details of the current cohort and the pilot group are presented separately before the results of the final analyses to demonstrate the comparability between these two groups.

2.4.1) Current Cohort

All brain trauma children (n = 76; Edinburgh n = 40; Newcastle, n=36) admitted enrolled between October 2000 to March 2003 were enrolled into the study. 74 of the cases were as a result of accidental traumatic brain injury, and 2 were suspected non-accidental head injury (one from each centre). Basic demographic data is tabulated below (Table 2.1).

Table 2.1:

DEMOGRAPHIC VARIABLE	n	OUTCOME VARIABLE	n
Gender:		Outcome (GOS) at 6 months post injury:	
Males	56	Dead	5
Females	20	Vegetative	0
Severity of Injury:		Severe Disability	4
Severe (GCS \leq 8)	46	Moderate Disability	35
Moderate (GCS = 9 -12)	27	Good Recovery	30
Mild (GCS 13-15)	3	Not known	2
Cause of Injury:			
Car occupant	6		
Motor Cycle driver	2		
Bicycle	7		
Pedestrian	31		
Short Fall <3'	9		
Fall from Height	10		
Struck on head	6		
Assault	1		
Sport (rugby)	1		
Penetrating Injury	1		
NAHI	2		

2.4.1.1) Physiological Data

Since the clinical needs of the patient dictated which physiological parameters were monitored during the intensive care admission, not all possible variables were monitored in every child. All time-series data that were monitored were collected and downloaded for analysis. For the 76 children, arterial blood pressure monitoring was collected in 74 cases, intracranial pressure monitoring in 52, heart rate in 75, core temperature in 63 and oxygen saturation via a pulse oximeter in all 76 children. As $CPP = MAP - ICP$, CPP values were available for 51 cases.

The mean time from injury to commencement of physiological monitoring was 10.7 hours, and the mean duration of monitoring was 124.28 hours (i.e. more than 5 days).

2.4.1.2) Comparison with Pilot Group

There were no statistical differences between the current and pilot groups for the following: gender, severity of injury, mode of injury, time to start of monitoring, percentage duration of derangement: for raised ICP, hypotension, hypertension, cerebral perfusion pressure, hypoxia, pyrexia and brady- and tachy-cardia.

2.4.2) Combined Current and Pilot Groups Data Set

For the total 128 children in this combined cohort, the descriptive statistics are provided below (Table 2.2) showing the numbers with and without derangements, the total monitored time, total derangement durations (minutes) and number of episodes of derangement for each parameter.

Table 2.2:

Variable	Number with derangements	Total duration (mins) of valid Monitoring	Total duration (mins) of derangements recorded	Range of valid monitoring time (mins)	Mean Duration of derangements (mins)	Median Duration of derangements (mins)	SD	Range of Insult durations (mins)	Median Number of episodes of derangement
Raised ICP	96/99	493241	391466 (79.4%)	97 - 17779	3954.2	2645	4117.8	0 - 17364	10
Hypotension	38/123	698911	4926 (0.7%)	44 - 42662	40.05	0.00	127.39	0 - 868	0
Hypertension	111/123	699710	307697 (43.9%)	44 - 42662	2501.6	1261.0	3207.91	0 - 18815	12
Low CPP	77/98	472136	72910 (15.4%)	0 - 17556	736.46	247.0	1296.5	0 - 9045	6
Hypoxia	23/124	769467	1951 (0.25%)	21 - 42909	15.73	0.0	94.17	0 - 1010	0
Pyrexia	75/109	461885	121209 (26.2)	120 - 29638	1112.01	475.0	1844.65	0 - 10159	1
Tachycardia	99/125	769627	164208 (21.3%)	97 - 42845	1313.66	265.0	2854.97	0 - 22284	4
Bradycardia	54/125	769431	53099 (6.9%)	97 - 42845	424.79	0.0	1078.19	0 - 5520	0

Overall, more than 5 million minutes (5 134 408) of data were collected. Derangements were found in every physiological variable, and every child was found to have some age-specific derangement in at least one physiological variable. The most commonly identified abnormality was raised intracranial pressure (in 97% of children), followed by hypertension (90%), low cerebral perfusion pressure (79%) and tachycardia (79%), pyrexia (69%), bradycardia (43%), hypotension (31%) and hypoxic derangements (19%).

The percentage duration of age-specific derangement per variable ranged from almost 80% for raised ICP to as little as 0.25% for hypoxia. The total duration of low cerebral perfusion pressure, which occurred in 15% of the valid monitoring time was clearly predominantly due to ICP being above threshold levels, rather than arterial hypotension which was recorded for just 0.25% total duration derangement.

2.4.3) Determining the Best Predictors of Outcome

Univariate logistic regression was carried out from the total of 128 patients, for both outcome groups: ISS, pupil reactions, presence of coma, GCS sumscore, RTS, TRISS, percentage duration of ICP and CPP were all significant for both dead/alive and poor vs. independent outcome. Tachycardia was found to predict mortality i.e. dead/alive ($p=0.047$) while hypertension predicted poor vs. independent outcome ($p=0.045$).

2.4.3.1) Those with ICP/ CPP Monitoring

99 children had intracranial pressure monitoring but in 12 patients, the ICP monitoring commenced 24 hours after the initial injury (range from 26.0 – 107.3 hours post injury) and they were, therefore, excluded from analyses. Additionally, one child had only non-invasive blood pressure monitoring, which could not be used to calculate CPP, so this case also had to be excluded from this analysis. 86 children, all with 6 months outcome data available, were, therefore, included in the final logistic regression modelling.

This final group comprised of 25 girls and 61 boys. Two children suffered a mild injury with ISS>16, 19 had moderate injury severity, and 65 with severe injuries on admission. At 6 months post-injury, 11 had died, there were no children who remained in a persistent vegetative state, 6 suffered severe disability, 33 were moderately disabled, and 36 made good recovery. Outcome groups were therefore Alive (75) vs. dead (11), and Independent outcome (69) vs. poor outcome (17).

2.4.3.1.1) Alive vs. Dead

Significant (univariate logistic regression) demographic features for outcome (Alive vs. Dead) at 6 months post injury (n=86), are given in Table 2.3.

Table 2.3:

Demographic Variables	p Value	Details	Odds Ratio	95% C.I.
Pupil Reactivity	0.045	Some Reaction No Reaction	5.12 1	(1.04 – 25.83)
GCS sumscore	0.024	Linear	1.53	(1.06 – 2.20)
Motor Score	0.012	Linear	1.97	(1.16 – 3.33)
RTS	0.004	Linear	2.66	(1.37 – 5.18)

Similarly, significant (univariate logistic regression) physiological derangement calculated as a percentage of the ‘acute monitoring time’ and outcomes (alive vs. dead) at 6 months post injury are shown below (Table 2.4):

Table 2.4:

Physiological Variables	p Value	Odds ratio	95% CI	Area under the ROC
Raised ICP	0.044	0.91	(0.84 – 0.99)	0.818
Low CPP	<0.0001*	0.94	(0.92 – 0.97)	0.890

With the data truncated to ‘acute monitoring time’ (i.e. duration of monitoring with ICP monitor in situ), tachycardia was no longer significant in the dead vs. alive comparison, and was therefore not included in the final model. The **final model (Logistic Regression, Forward step method)** for duration as a percentage of ‘acute monitoring time’ found the following for the alive vs. dead comparison (Table 2.5):

Table 2.5:

Variables	p - Value	Odds ratio	95% CI
Low CPP	0.000**	0.94	(0.92 – 0.97)
Raised ICP	0.088		
RTS	0.114		
Pupil Reaction	0.204		

2.4.3.1.2) Poor vs. Independent Outcome

Significant (univariate logistic regression) (n=86) demographic features for outcome (poor vs. independent) at 6 months post injury were summarised in Table 2.6.

Table 2.6:

Demographic Variables	p Value	Details	Odds Ratio	95% C.I.
Pupil Reactivity	0.038*	Some Reaction No Reaction	4.92 1	(1.09 – 22.26)
GCS sumscore	0.002**	Linear	1.77	(1.24 – 2.52)
Motor Score	0.000**	Linear	2.51	(1.51 – 4.15)
RTS	0.000**	Linear	3.09	(1.65– 5.80)

Similarly, significant (univariate logistic regression, n=86) physiological derangements calculated as a percentage of the ‘acute monitoring time’ and

outcomes at 6 months post injury (poor vs. independent) are summarised in Table 2.7.

Table 2.7:

Physiological Variables	p Value	Odds ratio	95% CI	Area under the ROC
Raised ICP	0.017	0.96	(0.92 – 0.99)	0.738
Low CPP	0.001*	0.96	(0.94 – 0.98)	0.793

With the data truncated to ‘acute monitoring time’, hypertension was no longer significant in the independent vs. poor comparison, and was therefore not included in the final model.

Final Model: Independent vs. Poor outcome, Logistic Regression. Forward step method for duration as a percentage of ‘acute monitoring time’. From total of n=86, 85 cases were in the final model (one with no MAP). (Table 2.8)

Table 2.8:

Variables	p Value	Odds ratio	95% CI
RTS	0.005*	2.65	(1.35 – 5.22)
Low CPP	0.014*	0.97	(0.94 – 0.99)
Raised ICP	0.189		
Pupil Reaction	0.556		

2.4.4) PTI Analyses

Of the 86 children with ICP monitoring, five were excluded from CPP quantification using the cumulative pressure time index because some CPP data were lost during the first 24 hours post injury due to computer downtime. Based upon the post resuscitation summated Glasgow Coma Score (GCS), sixty-three children of the 81

were considered to have suffered a severe head injury (GCS 3-8, E1, V \leq 2, and M \leq 5) and 16 children suffered a moderate head injury (GCS 9-12). There were two with a mild head injury (GCS13-15, with ISS \geq 16). The mean Injury Severity Scores was 20 (range 9 – 38).

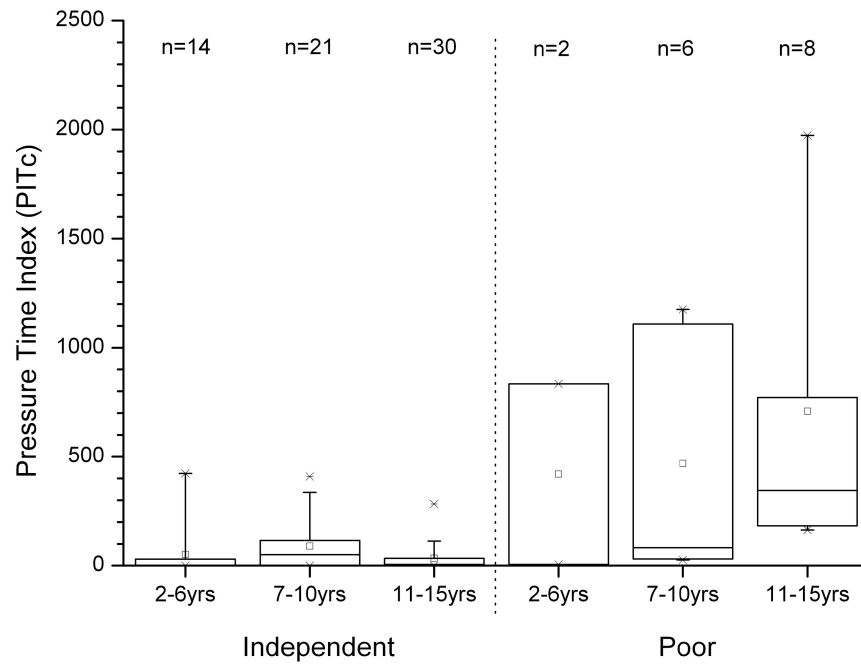
From the 81 children, 35 made a good recovery, 30 suffered from moderate disability (i.e. 65 had an independent outcome), 5 had severe disability at six months post injury and 11 died (i.e. 16 had a poor outcome). None remained in a persistent vegetative state.

2.4.4.1) PTI for CPP (PTIc)

Using the initial age-specific threshold values, the PTIc ranged from 0 – 1959 mmHg-hrs. The median PTI values varied significantly with Glasgow Outcome Score (good recovery 4.2; moderate disability 16.5; severe disability 73.6 and dead 769.1 mmHg-hrs, Kruskal-Wallis $p < 0.001$). There was no significant difference in the mean values of PTIc between the three age groups taken as a whole (not subdivided by outcome) i.e. comparable amounts of insult were found in all childhood age groups.

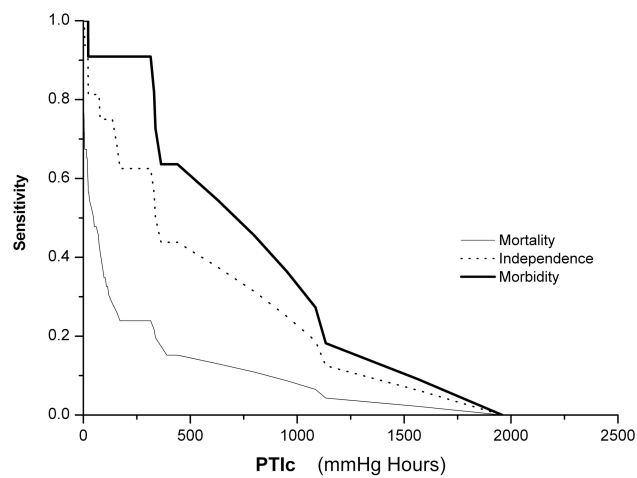
For both independent and poor outcome, there were no significant differences in the magnitude of PTIc across the 3 age bands (Figure 2.2, $p = 0.3$). However there was a very significant difference between the PTIc values in the poor vs. independent outcome categories ($p < 0.001$).

Figure 2.2:



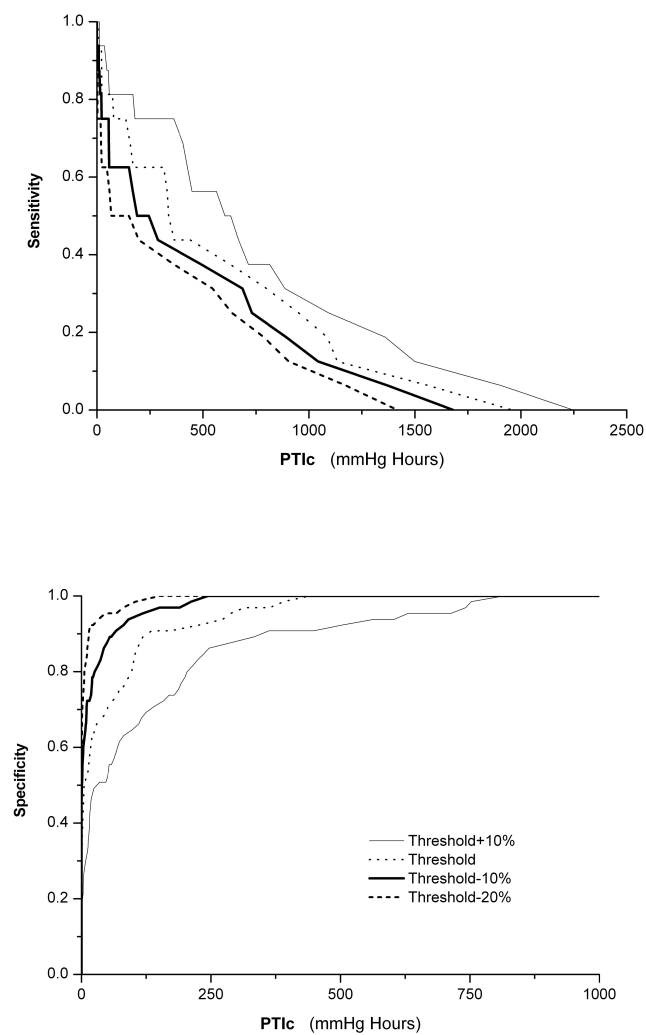
The PTIc values associated with an 80% sensitivity for independent outcome was 73.1 mmHg-hrs. The corresponding values for mortality and morbidity were 331 and 1.4 mmHg-hrs respectively (Figure 2.3).

Figure 2.3:



When the threshold levels were changed (increased by 10% and lowered by 10% and 20%), the sensitivity fell more rapidly with lower threshold values, but there remained a clear delineation between each of the curves of sensitivity (Figures 2.4). For a specificity of 80%, the PTIc values for each of the outcome groups were poorly separated at 99.5, 96.9 and 101.4 mmHg-Hrs for mortality, independence, and morbidity respectively, and with reduction in threshold values, the patterns were almost identical.

Figure 2.4:



The ROC curves for PTIc created using the initial CPP threshold values for the three different outcome dichotomies are shown in Figure 2.5 and the areas under the curves (AUCs) for all the ROC curves, along with their standard errors, are tabulated in Table 2.9. The PTIc index has a very high predictive value for mortality (AUC = 0.957) and for independent outcome it is only slightly lower (AUC = 0.890). The predictive power is the lowest for separating the ‘best’ recovery (i.e. GOS 5) from all the remaining outcome categories i.e. morbidity (AUC=0.681).

Figure 2.5:

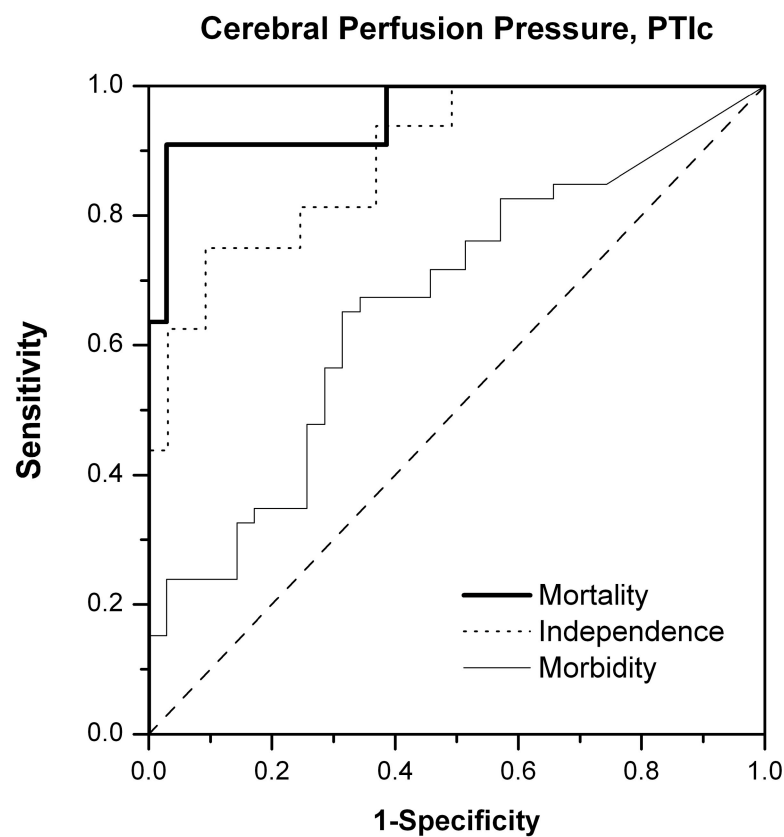


Table 2.9:

Outcome dichotomy	Threshold level	ROC area	Std. Error
Independent versus poor			
	CPP + 10%	0.858	0.054
	CPP	0.890	0.044
	CPP - 10%	0.883	0.053
	CPP - 20%	0.886	0.051
'Best' versus rest			
	CPP + 10%	0.652	0.062
	CPP	0.662	0.061
	CPP - 10%	0.645	0.061
	CPP - 20%	0.667	0.060
Mortality versus alive			
	CPP + 10%	0.931	0.046
	CPP	0.957	0.034
	CPP - 10%	0.968	0.027
	CPP - 20%	0.966	0.026

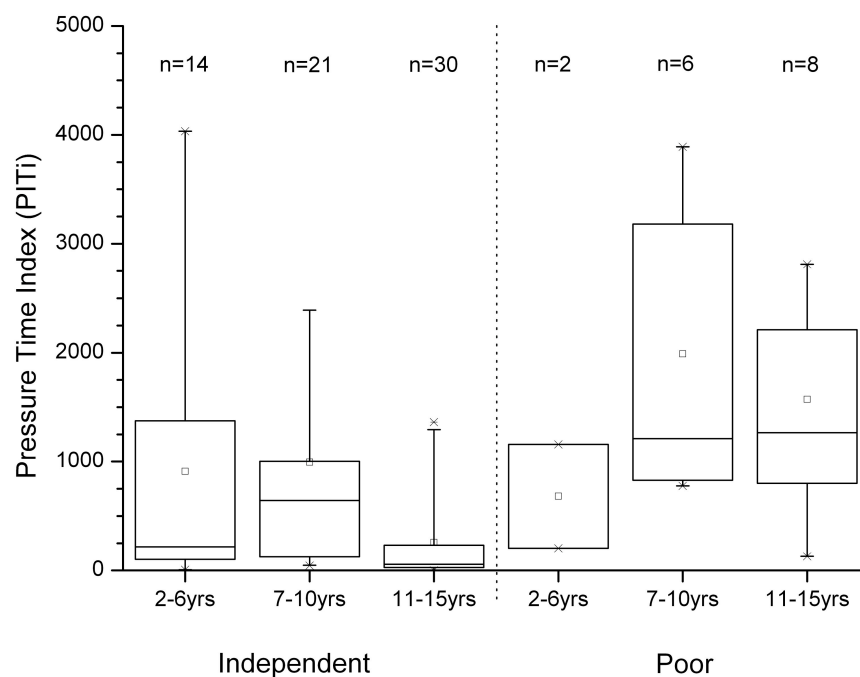
When the threshold levels were altered by the previously described amounts, the resultant ROC curves for each of the three different outcome comparisons had smaller AUC values than that of our original threshold, indicating our original thresholds were better predictive values (Table 2.9).

For independent outcome, the original CPP threshold remained the best predictor of outcome, but for both mortality and morbidity the predictive value increased very slightly (without reaching statistical significance) as the threshold level was decreased (Table 2.9).

2.4.4.2) PTI for ICP (PTIi)

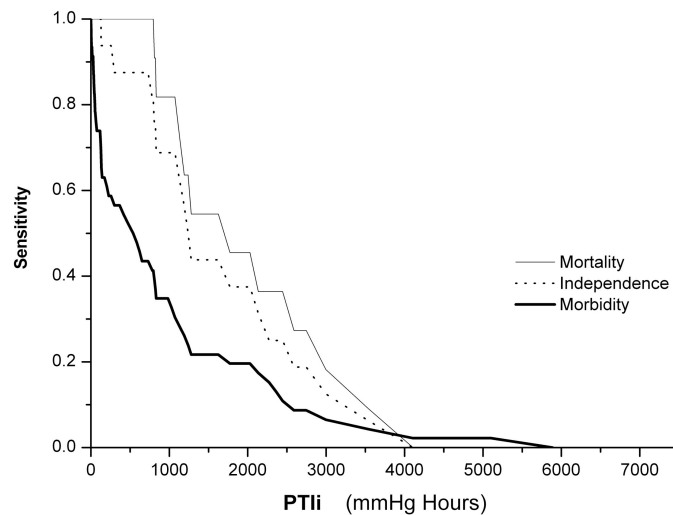
The results for PTIi were similar but not identical when compared to those for PTIc. The range of PTIi values was 0 - 5887 mmHg-Hrs, with a significant difference of median values of 232.8, 134.1, 776.3, and 1763.6 mmHg-Hrs in those who had good recovery (GOS 5), were moderately disabled (GOS 4), severely disabled (GOS 3), or died (GOS 1) respectively (Kruskall-Wallis, $p < 0.001$). There was a greater variation in the index across the two older age groups for both independent and poor outcome groups than for PTIc ($p = 0.026$ and $p < 0.001$) (Figure 2.6). There was a significant difference in the mean PTIi for independent vs poor outcome ($p < 0.001$).

Figure 2.6:



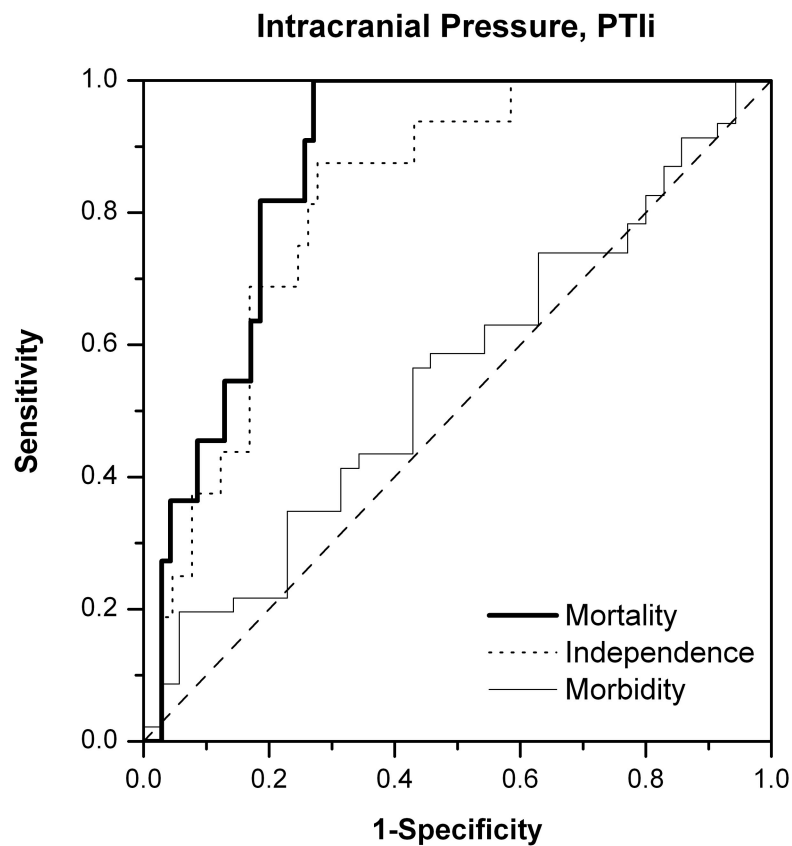
The rate of change of *specificity* was very similar to that of the CPP index for the 3 *outcome* comparisons. Although the shapes of the sensitivity curves were similar to those of PTIc, they were spread over a much wider range of values (Figure 2.7). For each of the 3 outcome dichotomies, the *different threshold* levels produced very similar sensitivity and specificity curves with little difference between mortality, independence and morbidity values, unlike those for CPP.

Figure 2.7:



The areas under the ROC curves for the PTIi for mortality, independent outcome, and morbidity were smaller than the respective PTIc values (Figure 2.8 and Table 2.10). The PTIi index has a very high predictive value for mortality (AUC = 0.871) and a slightly lower value for independent outcome (AUC= 0.819). For morbidity, ROC curve stays near to the diagonal indicating that the predictive value was always close to 50%.

Figure 2.8:



The final ICP values (i.e. ICP values before ICP monitoring was discontinued) for children aged 2-6 were 12 mmHg, for 7-10, 18mmHg, and 11-15 years, 16 mmHg, corresponding final CPP values (i.e. CPP values before ICP monitoring was discontinued) of 78, 74 and 79 mmHg respectively. Although 42 children had final ICP values that were a mean of 11 mmHg above their age-specific thresholds, this was obviously compensated because there was a negligible amount of corresponding final CPP insult, with only 5 children having had final CPP values of a mean of 3 mmHg below threshold levels. Even if this level of insult in these 5 patients continued for the next 1 hour, the PTI would still have estimated 98.6% of all CPP

insult. It can be seen, therefore, that the pressure recordings has been discontinued only when there was virtually no ongoing CPP derangement.

Table 2.10:

Outcome dichotomy	Threshold level	ROC area	Std. Error
Independent versus poor			
	ICP - 10%	0.816	0.050
	ICP	0.819	0.050
	ICP + 10%	0.823	0.050
	ICP + 20%	0.825	0.052
'Best' recovery versus rest			
	ICP - 10%	0.555	0.065
	ICP	0.550	0.065
	ICP + 10%	0.548	0.064
	ICP + 20%	0.545	0.064
Mortality versus alive			
	ICP - 10%	0.864	0.042
	ICP	0.871	0.041
	ICP + 10%	0.882	0.039
	ICP + 20%	0.887	0.038

The duration of ICP monitoring bore no relation to the severity of the primary injury as assessed by the initial GCS motor score level ($p = 0.572$), nor to the Injury Severity Score ($p = 0.237$). In addition, there was no significant difference between the two participating children's neuro-intensive care units in the duration of ICP monitoring ($p = 0.749$).

2.5) DISCUSSIONS

This study examined the relationships between different age-specific physiological derangements and outcome in critically ill children following traumatic brain injury. Inadequate cerebral perfusion pressure by age was demonstrated to be the best physiological parameter routinely monitored in the ICU to predict outcome. Furthermore, successful development of a bi-dimensional index to summarise the total burden (i.e. duration and magnitude) of age-related cerebral perfusion pressure insult has enabled critical age-related cerebral perfusion pressure thresholds to be defined.

2.5.1) Physiological Derangements

Intensive search for effective neuroprotective strategies to improve outcome after brain trauma has been disappointing. The current best treatment option available for these critically ill patients remains the detection and prevention of secondary brain insults from physiological derangements. Avoidable secondary physiological derangements may occur at any stage after brain trauma [64-69] and those that are known to correlate with poorer outcome include hypoxia, hypotension, raised ICP, low CPP, or pyrexia [72, 92]. However, it is unclear from the literature which of these abnormal physiologies best correlate and predict outcome.

Detection and prevention of physiological derangement rely heavily upon accurate monitoring. Continuous physiological monitoring in minute resolution has become

the expected routine practice in modern neuro-intensive care for nearly three decades. However, data collection from previous brain trauma studies of critically ill patients rarely utilise recording with this degree of accuracy despite development of research computer systems that are capable of capturing, recording, and analysing the continuous physiological data to identify and quantify potential insults. Majority of these studies used end-of-hour data to assess the relationships between potential physiological insults and outcome. However, physiological derangements may occur at any time during the intensive care management and may not have been present during the collection of the end-of-hour data. Using end-of-hour data, therefore, poses a great danger in omitting potential insults and the true relationship between physiological insults and outcome cannot be determined. Using real time physiological data, all potential insults would be included in the analyses thereby increasing the accuracy of insult quantification and a more valid conclusion may then be drawn on their relationships with outcome.

In this current study of the critically ill brain injured children, more than 5 million minutes of data were collected. Physiological derangements were identified in all children but not all parameters were necessarily deranged in every child. Given the composition of our cohort is similar to other previously published British and European childhood TBI case series with respect to the type, cause and severity of injury as well as outcome [4, 7, 48, 197, 198], our findings have highlighted that secondary physiological derangements occur extremely frequently in children after TBI during the intensive care management. The accuracy of data collection and the amount of data available in this current study have also enabled us to confirm that

age-specific cerebral perfusion pressure derangement was the best predictor of outcome in childhood brain trauma.

2.5.1.1) CPP and ICP Derangement

2.5.1.1.1) CPP Derangement

2.5.1.1.1.1) Incidence and Duration

Comparison of the incidence and duration of CPP derangement in children after TBI within the literature remains difficult partly because of the lack of normative data by age making definition of derangement variable between studies, and ICP monitoring is not a uniform practice in neuro-intensive care worldwide. Chambers and colleagues examined a total of 4373 hours of CPP monitoring collected prospectively from 84 children during their intensive care treatment following TBI and found that CPP derangement (i.e. recording below their single pre defined threshold of 70 mmHg) was present in 2083.7 hours of the monitoring time (47.6%) [91]. This figure was 3.2 times higher than the previously reported overall duration of CPP derangement of a group of adult brain trauma patients from Edinburgh using the same single intervention threshold [72]. Injury severity of the cohort reported by Chamber and co-workers [91] was not more severe than other previously reported childhood brain trauma cohorts and therefore, could not account for the longer duration of CPP derangement identified. Despite having apparently longer CPP derangement, 75% of their cohort had recovered with a good recovery and the

mortality rate was 13% [91] which was similar to the expected mortality for brain injured children in the UK and other European countries [1, 3, 8-10, 12, 14-16, 20, 21, 25, 26, 46-49]. The threshold for CPP derangement in Chambers' report [91] was the same as the widely accepted adult CPP threshold [74] and, therefore, may not accurately identify the true frequency of CPP derangement in children after brain trauma given that the CPP thresholds in normal children have been generally agreed to be below 70 mmHg [184, 188-190].

Jackson and co-workers determined the incidence and duration of CPP derangement in a cohort of 18 brain injured children aged less than 16 years of age by applying three arbitrary chosen CPP thresholds: $CPP < 70$ mmHg, $CPP < 60$ mmHg, and $CPP < 50$ mmHg [90]. They found that 30% of the CPP monitoring time of these children were between 60 and 70 mmHg [90], which was similar to the figure from the much larger cohort reported by Chamber and colleagues [91]. However, Jackson's group found that as the CPP threshold was reduced arbitrary to less than 60 and 50 mmHg respectively, lesser duration of the CPP recording was identified as being potential derangement (21% and 8% of the monitoring time were respectively detected as potential derangement with a threshold of > 60 mmHg and > 50 mmHg) [90]. This has confirmed the speculation that more false positive CPP derangement would be identified in children after brain trauma by using super-normal CPP thresholds than using thresholds closer to the physiological norm since most authors agreed that normal CPP in infants and children should be between 40 and 60 mmHg [184, 188-190]. The CPP thresholds chosen by Jackson and co-workers were, however, without physiological basis and they applied these thresholds to the whole group

without considering any age maturation effect on the normal CPP physiology [90]. Thus, age related CPP thresholds are required to determine the true incidence and duration of CPP derangement in children.

A previous report of our pilot data by Jones and colleagues indicated that 77% of brain injured children had age specific CPP derangement during intensive care treatment and 11.8% of the total duration of CPP monitoring was lower than the pre defined age specific thresholds i.e. potential derangements [92]. Using the same theoretical age specific levels to define CPP derangement, our current study which included the original pilot data identified 1215.2 hours of CPP derangement out of a total of 7868.9 hours of CPP monitoring (i.e. 15.4%). Thus, 42% less potential derangement was found in our current study when compared to those identified by Chamber's group [91] despite having more prolonged period of monitoring and using narrower but age-specific thresholds to define abnormal physiology in our current study. Given the different thresholds used throughout childhood, the overall duration of CPP derangement identified in our cohort was also comparable to those previously reported in adults after brain trauma [72].

2.5.1.1.1.2) Causes of CPP Derangement

CPP derangement may be the direct results of raised ICP alone, arterial hypotension alone, or a combination of the two since CPP is calculated through the formula:

$$\text{CPP} = \text{MAP} - \text{ICP}$$

Cortbus and colleagues reported on the causes of episodes of reduced cerebral perfusion pressure in a group of 74 adults with traumatic brain injury [199]. Two clusters of reduced CPP were found with one cluster occurring during the first 24 hours of monitoring and the other at 5 or 6 days after injury [199]. The causes of reduced CPP between these two clusters were very different, with arterial hypotension being the predominant cause for the first cluster while intracranial hypertension was responsible for majority of the reduced CPP during day 5 or 6 post injury [199]. The latter finding did not raise any surprises because raised ICP had been demonstrated to occur frequently after brain trauma [63, 70] and cerebral oedema usually worsen over the first few days after the initial primary brain injury. However, arterial hypotension related reduced CPP during the acute management of these patients would suggest that resuscitative measures for these patients were less than optimal.

Chambers and colleagues also investigated the causes of secondary insults in 84 children and 291 adults after brain trauma and found that secondary insults occurred regardless of the outcome [91]. Using adult threshold values for ICP, CPP and arterial blood pressures (MAP and systolic blood pressure), they demonstrated that majority of the CPP insults in children were caused by decreased arterial blood pressure and as the thresholds used to define low CPP is reduced from 70 mmHg to 60 and then 50 mmHg, the number of insults caused by a combination of reduced arterial blood pressure and raised ICP were increased [91]. Furthermore, almost all insults were due to a combination of reduced arterial pressure and raised ICP when CPP were less than 50 mmHg in their paediatric cohort [91]. These findings differ

significantly from those of our current study since the predominant cause of low CPP in our patients was raised ICP rather than arterial hypotension which only occurred in 0.25% of the total monitoring time throughout the study. This highlights the importance and the need to consider the age-maturation effects on the intracranial and systemic pathophysiology when conducting paediatric brain injury studies and appropriate age related thresholds should be used to define secondary physiological insults in childhood brain trauma.

2.5.1.1.2) ICP Derangement

Raised ICP was the most frequently identified derangement in our study and was found in virtually all cases (97%). Mayer and Walker had previously reported in 1985 that intracranial hypertension, which was defined as having a raised ICP of more than 20 mmHg, occurred in 80% of the 200 children they investigated after brain trauma [200]. The incidence of intracranial hypertension was 1.2 times higher in our current cohort recruited 15 years later than those described by Mayer and Walker [200]. Treatment of raised ICP has changed little over the years, but continuous computerised physiological monitoring is now the norm in modern neuro-intensive care and the accuracy of data collection used in our study may explain the increased detection of intracranial hypertension in our cohort. Different thresholds were used to define intracranial hypertension between our current study and that of Mayer and Walker's [200]. The thresholds used in our study to define raised ICP were theoretical age-specific norms and were much lower than thresholds definition of intracranial hypertension that were used by Mayer and Walker [200]

and may explain the discrepancies between the frequencies of raised ICP reported by these two studies.

Mayer and Walker were not the only investigators to apply an adult ICP threshold to define raised ICP in brain injured children because of the lack of normative ICP values by age. Brain trauma children with ICP more than the widely accepted adult threshold of 20 or 25 mmHg were associated with poorer outcome or death. Pfenninger and co-workers retrospectively reviewed 24 children after brain trauma who were managed with a treatment goal of maintaining ICP of less than 20 mmHg and to abolish persistent ICP rises of more than 25 mmHg for 3 minutes [201]. They found that persistently raised ICP of more than 40 mmHg was significantly associated with death [201]. In another retrospective review of 56 children with severe TBI, Esparza and colleagues similarly found a significantly higher mortality among brain injured children whose ICP were more than 40 mmHg which was 3.6 times higher than those who had ICP between 20 and 40 mmHg (100% vs. 28%) [202]. Both of these studies [201, 202] were conducted retrospectively and although they had highlighted the obvious association between prolonged periods of raised ICP and poor outcome after brain trauma, no attempts was made to address ICP treatment thresholds or to define the physiological changes in ICP with age.

The relationship between intracranial hypertension and cerebral blood flow post brain trauma among 21 severely brain injured children was assessed prospectively by Sharples' group [89] who also defined intracranial hypertension as having an ICP elevation of more than 20 mmHg for 10 consecutive minutes. This important report

suggested for the first time that cerebral hyperaemia was an uncommon cause of intracranial hypertension in paediatric TBI because cerebral hyperaemia occurred only in 2 out of 21 children they examined [89]. However, like other paediatric brain injury studies, the definition of intracranial hypertension was not age adjusted. Given that majority of authors agreed that the normal ICP for children would be much lower than the traditional thresholds of 20 mmHg, it is possible that by applying adult ICP thresholds to child cohorts, the frequency and duration of true age-related intracranial hypertensive insult is under-estimated. Our study has, therefore, highlighted the importance of applying age-related thresholds when investigating childhood brain trauma.

Despite the possibility of underestimating the frequency and duration of true intracranial hypertensive insults, these previous reports have led to the general acceptance of raised ICP being a secondary physiological insult that adversely affects outcome after paediatric brain trauma. Treatment of raised ICP of more than 20 mmHg after TBI has been recommended as a treatment option for intracranial hypertension in the recently published first edition of the Guidelines for the Acute Medical Management of Severe Traumatic Brain Injury in Infants, Children and Adolescents [75]. This guideline has emphasized the insufficient evidence in the current literature to allow formulation of treatment standards or even guidelines. It was, therefore, not possible to recommend specific thresholds for treatment of intracranial hypertension in infants and children after TBI. It is, therefore, of paramount importance to direct future research focus in defining critical age related treatment thresholds. Using theoretical physiological norms for ICP adjusted with

age as thresholds, the predictive value of the age adjusted ICP derangement (as a percentage of the acute monitoring time) on the mortality and morbidity after childhood brain trauma was demonstrated for the first time by our current study. We have, therefore, presented a novel approach to define ICP derangement by age. However, as the thresholds used in our study were only theoretical physiological norms and may not necessary represent insult thresholds, there is an urgent need to define age-specific ICP insult thresholds so that treatment thresholds may be developed.

2.5.1.1.3) Quantification of the Total Burden of Secondary Intracranial Physiological Insults

Development of computerised data collection and analysis systems have progressed dramatically over the past decade enabling better identification of potential secondary physiological insults. However, previous studies of the relationships between CPP and outcome in both adult and childhood brain trauma had focused on using a single measurement of CPP derangement values (either mean, minimum, absolute, or percentage duration) but all failed to incorporate the effect of both the duration and severity in the total burden of CPP insults. The failure to accurately quantify the totality of CPP derangement may have limited the determination of critical ICP and CPP treatment thresholds, and development of novel therapies. This study is the first to address this problem by successfully creating a novel pressure time index that simultaneously measure both the duration and intensity of CPP

derangement (i.e. quantifying the total burden of abnormal physiology) using detailed physiological data [203, 204].

The pressure time index was a measure solely of secondary brain insult and allowed comparability of insults at any age [203, 204]. Insult comparability across different ages is of particular importance in the progress of future paediatric TBI studies given the urgent needs to investigate the age maturation intracranial physiological variations between infants and children of different ages. With the development of the pressure time index, this study has also derived critical CPP insult thresholds in three paediatric age groups and confirmed the importance of CPP as a determinant of outcome in childhood traumatic brain injury [204].

2.5.1.1.3.1) Cumulative Pressure-Time Index for CPP (PTIc)

The PTIc values were significantly related to each of the GOS outcome categories or the combinations we had used (independent vs. poor, mortality, morbidity) [204]. Other studies had shown a similar relationship of ‘single dimension’ CPP derangement to outcome [91, 92]. The similar PTIc values across our three different age bands indicated that this novel index was independent of age thereby allowing comparability of CPP insults at any age [204].

The sensitivity of the PTIc (i.e. proportion of those who made an independent outcome reduced with increasing PTIc) – separated the three outcome comparisons showing that the largest insult burden occurred in those who died, and the least insult

was found among those who made a good recovery [204]. Specificity, on the other hand (i.e. the proportion of poor outcome patients increased with increasing values of the PTIc) proved to be less discriminating for our outcome categories, and remained so when the theoretical thresholds were applied [204].

Receiver Operator Characteristic (ROC) curve is a plot of sensitivity against 1 – specificity. It is a powerful tool for evaluating a process that has a binary outcome without the need to define one single arbitrary cut-off level [205]. It provides a simple and easily interpretable graphical display of the trade-off between sensitivity and specificity at each decision threshold [205]. Furthermore, the area under the ROC curve (AUC) gives a measure of the predictive value of a test and can be used to compare different variables [206], with an AUC of 1.0 equating to perfect prediction while that of 0.5 indicating random chance.

The threshold levels that were chosen were based upon hypothetical minimum physiological norms, (48 mmHg for those aged 2 – 6 years; 54 mmHg for those aged 7 – 10 years; and 58 mmHg for those aged 11 – 15 years) and were not known at the outset to be insult thresholds. We reduced the CPP thresholds arbitrarily by a factor of 10 and 20% and similarly increased it by 10% (theoretical values), and compared these sensitivities and specificities. Our ROC analysis had clearly demonstrated that the PTIc values for CPP calculated using our original theoretical CPP threshold values at different ages most accurately separated the outcome categories [204]. Altering these theoretical threshold levels failed to improve the predictive value

indicating that the original physiologically based theoretical thresholds were in fact critical insult thresholds for CPP [204].

2.5.1.1.3.2) Cumulative Pressure Time Index for ICP (PTIi)

Although it is generally accepted that raised intracranial pressure can promote deleterious events that can be related to outcome, previous studies of ICP and outcome in the brain trauma literature have failed to demonstrate any statistic significance. For ICP, the high ROC area under the curve values for mortality and for independent vs. poor outcome using our new index indicated for the first time such a considerable relationship to outcome [204]. However, its predictive value remained less than that of the PTI for CPP highlighting once again the importance of maintaining an adequate CPP during the acute management of these patients [204].

Children who were severely disabled or died in our cohort had progressively larger amounts of ICP insults than those who had moderate disabilities or good recovery as would be expected. However, children with good recovery appeared to have suffered more ICP insult than those with moderate disabilities. The duration of monitoring did not differ significantly between patients with good recovery and those with moderate disabilities. One possible explanation of this anomalous finding is that the pre-defined theoretical age-related ICP thresholds were not critical insult thresholds. Alternatively, other factors, such as the genetic constitutions of the patients, might have different influence on how they responded and recovered from raised ICP, thereby affecting outcome differently. Another possible explanation is

that cerebral autoregulation remained intact in patients with good recovery and as a result, they were able to compensate the higher burden of ICP insult by maintaining an adequate cerebral perfusion pressure. This highlighted the importance of examining cerebral autoregulation in brain trauma patients in future studies.

Increasing or decreasing the ICP threshold did not significantly change the sensitivity or specificity and hence did not improve the predictive value in relation to outcome – i.e. it is the CPP at our age-specific thresholds that is the more influential factor in the outcome of paediatric brain trauma [204].

2.5.1.1.3.3) Critical Thresholds

We divided our patients into three age groups to allow calculation of the PTI and determination of critical physiological thresholds but these age bands have subsequently been described in another report which used the hourly measurements of the first 6 hours of acute monitoring data from over 200 head injured children [207]. The ROC curves from our current study demonstrated differences in the specificity of CPP and ICP with outcome for the various age groups where at 50% specificity, the CPP values for the three age groups (age 2 - 6 years, 7 – 10 years, and 11 – 16 years) separated at 53, 58, and 67 mmHg; and those for ICP separated at 24, 19, and 18 mmHg respectively for the three different groups [204]. Although these bands could be refined, in practice the rate of development of the physiology of children can vary quite markedly and therefore the banding provides a measure of spread. We are conscious that we have not addressed ages between 0 and 2 years,

because the rapidly changing physiology at this stage of development would require a large cohort of patients and may only be possible with a large multi-centre study.

There are three different types of thresholds: (1) brain insult thresholds are threshold values where irreversible brain damage will occur if CPP is persistently below the critical CPP insult threshold or ICP is persistently above the critical ICP insult threshold; (2) physiological thresholds are threshold values where physiology is deranged but not necessarily causing irreversible tissue damage; and (3) treatment thresholds are thresholds where clinicians act and instigate treatment for raised ICP or low CPP. It is logical to think that brain insult thresholds are likely to be less than physiological thresholds and would require measurement of brain metabolism to define these insult thresholds. For treatment thresholds to be effective in improving outcome by preventing brain insults and physiological derangements, they would need to be above the physiological thresholds. We have tested our proposed age-related theoretical physiological thresholds for CPP and proved that they are in fact critical thresholds since lowering them further did not improve the predictive value of outcome [204]. Each of these needs to be precisely defined and understood, but we believe that for children, the CPP physiological thresholds are in fact, identical to the brain insult thresholds.

Current critical care guidelines for adult and childhood brain trauma have emphasized the usefulness of ICP monitoring in selected patient groups but, because of insufficient evidence, have failed to recommend universal ICP monitoring in all brain trauma patients requiring intensive care. With the newly defined age-related

critical CPP thresholds, the argument for using ICP monitoring should now be strengthened. Furthermore, this novel Cumulative Pressure-time Index is essential for future studies because the ability to quantify the totality of secondary physiological insults will enable ICP and CPP physiological / insult thresholds in adults to be defined. In addition, current intensive care treatment regimes may be validated, and critical care management options may be explored.

2.5.1.2) Other Derangement

2.5.1.2.1) Blood Pressure Derangement

2.5.1.2.1.1) Hypotension

Normative data on age related changes in arterial blood pressure is well documented in the literature [87, 181-183]. Unlike the definition of intracranial hypertension, majority of the studies in the literature assessing the relationship between hypotension and outcome in paediatric brain trauma have employed age related thresholds to define hypotension, with many studies adopting the widely accepted definition of having a systolic arterial blood pressure below the 5th percentile for age for 5 consecutive minutes.

The incidence of age adjusted hypotension in childhood TBI has been reported to be between 29 and 39%. In 1985, Mayer and Walker reported that 29% of patients among the 200 severely brain injured children they investigated had low arterial

blood pressure for their age [200]. Despite its less frequent occurrence in comparison to raised ICP in their cohort, hypotension was associated with a 2.5 times increased in mortality when present [200]. The significant influence of hypotension on the mortality after childhood TBI was confirmed by a later prospective study involving 1906 children and 6908 adults with severe brain trauma where the mortality of brain injured children aged less than 15 years with hypotension was 2.8 times higher than that of brain injured adults with hypotension [208].

Levin and colleagues investigated physiological data recorded prospectively from a cohort of 103 children with severe brain trauma who were recruited as part of the Traumatic Coma Data Bank study and found that younger children, aged 0 to 4 years, had higher incidence of hypotension and the poorest outcome [18]. Another review of the prospectively collected data of 75 children with severe brain trauma by the Trauma Registry confirmed that the presence of hypotension in the field or the emergency department were associated with higher mortality [209]. The importance of early hypotension (i.e. on the scene of the accident or in the Emergency Department) on brain trauma outcome was confirmed by another retrospective study of 93 brain trauma children attended a Level I Trauma Centre in the United State of America [210]. In 1993, Pigula and colleagues reported a 2.8 times increased in mortality in the presence of hypotension on arrival to the emergency department among a cohort of 58 severely brain injured children recruited prospectively over a 5 year period [179]. Kokoska and co-workers retrospectively reviewed medical charts of 72 brain injured children with moderate severity and found that 39% of patients

had hypotension while being resuscitated in the emergency department, and a similar proportion (37%) suffered hypotensive insults during intensive care management [211]. Patients in this cohort with moderate and severe disability had significantly more episodes of hypotension recorded than those who recovered with good outcomes [211].

Hypotensive derangement was found in 31% of the children in our current study but was only detectable in 0.7% (82.7 hours) of the total duration of monitoring time (over 11648 hours or 485 days). The incidence of hypotensive insults identified in our cohort is, therefore, similar to those previously reported suggesting that despite significant development in advance trauma care and acute resuscitation technique over the past decade, considerable proportion of children continued to suffer from hypotensive insults after traumatic brain injury. However, the duration of hypotensive derangement in our cohort was very low and was not statistically predictive of mortality or morbidity. This would suggest that although modern acute trauma and neuro-intensive care have made little impact on the incidence of hypotension over the last three decades among critically ill brain injured children, they have successfully reduce the duration of hypotension in these patients to break the association of hypotension with mortality as previously reported.

2.5.1.2.1.2) Hypertension

Arterial hypertension after brain trauma has not been as extensively investigated as other physiological derangements like intracranial hypertension, hypotension,

hypoxia or reduced CPP. The literature's definition of hypertension post paediatric TBI is not well defined and a conflicting relationship between arterial hypertension and outcome following childhood TBI has been reported by the few studies in the literature.

Kanter and colleagues examined the relationship between persistent arterial hypertension and outcome of 42 comatose children caused by brain trauma, Reye's syndrome, anoxia, and CNS infection [212]. They found that 74% of patients with high blood pressure persisting until ICU discharge had poor outcome [212]. It is possible that children included in this study had lost cerebral autoregulation, as a result, an increase in the arterial blood pressure would be transmitted intracranially resulting in a mirroring rise in the ICP thereby failing to improve the CPP which resulted in the poorer outcome. Alternatively, 'false cerebral autoregulation' may explain the inverse relationship between arterial hypertension and outcome. 'False cerebral autoregulation' is a concept described by a recent cerebral blood flow study in adults where the cerebral blood flow remained constant or even reduced during a clinically apparent improvement in the CPP with induced arterial hypertension despite a simultaneous rise in the ICP [213].

However, more recent studies have described a favourable association between arterial hypertension and outcome in paediatric TBI. Luerseen and colleagues reported that the lowest mortality was observed among brain injured children with severe hypertension which was defined as having a systolic blood pressure > 135 mmHg [208]. Similarly in another retrospective study which tried to determine

predictors of outcome among severely brain injured children, White and co-workers found that the odds of survival was increased by 19 folds with the present of a maximum systolic blood pressure of more than 135 mmHg [214]. It is unclear whether the reported super-normal blood pressure observed in children within these studies were artificially raised with inotropes or whether it was the result of the cerebral autoregulatory response. Nevertheless, it is easy to understand the association of an improved outcome with arterial hypertension because having a higher systolic blood pressure would result in a higher than normal mean arterial blood pressure which would in turn improve cerebral perfusion pressure in the presence of an intact cerebral autoregulation, thereby improving outcome. Thus, the association between super-normal arterial blood pressure and an improved outcome following childhood brain trauma should not pose any surprises.

Age adjusted arterial hypertension was detected in majority (90%) of our patients and had significant predictive value on good outcome. It is likely to be a reflection of the management guidelines used and their emphasis on the importance to maintain an adequate CPP after childhood brain trauma by raising the systemic arterial pressure with the use of inotropes. This finding once again highlighted the importance of maintaining an adequate cerebral perfusion pressure.

2.5.1.2.2) Hypoxia

Correlation between hypoxia and unfavourable outcome after brain trauma was first reported in 1958 when McIver and co-workers described the role of respiratory

insufficiency in the mortality of severely brain injured adults. Definitions of hypoxia varied between reports in the literature with older studies adopting cyanosis as a clinical diagnosis of hypoxia, while studies after the development of arterial blood gas analyses in 1966 using various thresholds between 60 and 80 mmHg to define hypoxia. Since it has been shown that an oxygen saturation of less than 90% generally equates to an arterial partial oxygen concentration of less than 60 mmHg, some investigators have opted to use the non-invasive pulse oximetry monitoring to define hypoxia with the thresholds varying between 90 and 92%.

Correlation between poorer outcome and hypoxia sustained at different stages of the management of adult TBI, such as at presentation to emergency departments, on admission to or while in intensive care or neurosurgical units, has encouraged the Brain Trauma Foundation to recommend vigilant prevention and prompt treatment of hypoxia after brain trauma as a 'treatment guideline' in their latest Guidelines in the Acute Management of TBI [74].

Paediatric evidence linking hypoxia and poor outcome after brain trauma is scanty with most of the reports concentrating only on the effect of hypoxia documented in the emergency department following brain trauma and the results between reports are often contradicting. Mayer and Walker first suggested hypoxia causing increased mortality in childhood brain trauma in 1985 when they prospectively examined 200 children with severe brain trauma and the determinants of poor outcome [200]. They found that patients with systemic complications such as hypoxia, hypercarbia, or hypotension identified in their emergency department had a mortality rate that was

seven times higher than those who did not experience any of these systemic complications [200]. However, they did not investigate the individual effect of these systemic complications on outcome. Hypercarbia is the single most potent inducer of intracranial hypertension while hypoxia and hypotension contribute to ischaemia, it was therefore, not possible to differentiate the individual contribution of these systemic complications to poor outcome from this report.

Subsequent reports describing the correlation between hypoxia and poor outcome after brain trauma have also grouped hypoxia with other systemic complications such as hypotension when assessing their effects on outcome. Sharple and co-workers reported a four fold increased in mortality from brain trauma caused by hypoxia among children with evidence of hypotension when they were examining potentially avoidable factors among fatal cases of paediatric brain trauma [59]. In a recent Italian prospective study assessing the pre-intensive care complications and their impact on outcome of brain injured children, Chiaretti and colleagues similarly found that early complications such as hypoxia and hypotension significantly related to poorer outcome regardless of the severity of the primary brain injury as measured by the GCS [215]. Chiaretti and co-workers conducted another study to retrospectively determine prognostic factors of childhood TBI and similarly reported a 5 fold increased in poor outcome after brain trauma in the presence of early complications (i.e. hypoxia or hypotension) occurring in the emergency departments [216]. However, only the combined effect of hypoxia and hypotension on outcome was assessed without determining the specific contribution from each of these early complications.

The lone effect of oxygenation on childhood brain trauma outcome were described by 3 groups of investigators. Ong and colleagues reported hypoxia being solely responsible for an increase in poor outcome after severe paediatric head injury [153]. They evaluated the prognostic value of hypoxia, hypotension, and measures of primary brain injury such as the GCS and the initial brain CT findings on outcome after paediatric head injury in Kuala Lumpur using a data set collected prospectively from 151 consecutive children admitted with head injury including those without evidence of brain trauma [153]. The 2 to 4 folds increased in poor outcome observed in the presence of hypoxia occurred independent of hypotension and other systemic complications [153].

Pigula and co-workers, however, reported that hypoxia on its own did not alter mortality after paediatric brain trauma [179]. They analysed single point data collected on admission to the emergency department from 509 brain injured children of which 58 were recruited from a single trauma centre in Vermont while the rest were data provided by the National Trauma Registry [179]. Hypotension was found to significantly increase mortality after brain trauma in children but combined hypoxia and hypotension only slightly increased mortality over the effect of hypotension on its own [179]. They concluded that hypotension was the more critical factor in oxygen delivery post brain trauma and hypothesized that the brain had significant oxygen extraction capacity to prevent ischaemia even in the presence of hypoxia but hypotension would impair oxygen delivery to the brain even in the presence of normal PaO₂ causing damaging ischaemic insults [179]. The difference

in findings between Ong's group and Pigula's might be explained by the different inclusion criteria and study end points. Ong's group examined the effect of hypoxia on outcome among head injured children of all severity regardless whether there was evidence of brain injury [153], while Pigula's group investigated mortality among children who had evidence of severe brain injury [179].

It is possible that hypoxia has more influence on morbidity than mortality. In a retrospective analysis of Trauma Registry data collected prospectively on 75 brain injured children, Michuad and colleagues found that hyperoxygenation was related to better outcome [217]. In their series, brain injured children with a PaO₂ of more than 350 mmHg in the emergency department had better outcome than those with a PaO₂ of 105 to 350 mmHg [217]. The arterial partial pressure of oxygenation quoted in their cohort even at the lowest level of 105 mmHg was still well above any recognised thresholds of hypoxia (60 to 80 mmHg). It is, therefore, impossible to stipulate whether hypoxia has a detrimental influence on outcome after paediatric brain trauma. But the findings did suggest that hyperoxygenation during resuscitation might confer a beneficial effect on outcome in childhood TBI. Modern trauma resuscitation and emergency care emphasizes on the priority to control and maintain the airway and breathing with the provision of high flow oxygen therapy and mechanical ventilation to prevent hypoxia and hypercapnea respectively while hypotension is promptly identified and being corrected.

Little information is available in the literature on the frequency of hypoxia in the intensive care setting following paediatric brain trauma. Hypoxia was extremely

uncommon among our patients during their intensive care management reflecting the success of modern trauma care.

2.6) MONITORING OF BRAIN OXYGEN AND SUBSTRATE DELIVERY & CONSUMPTION

Assessment of brain oxygen and substrate delivery and consumption has gained considerable interests in adult brain trauma research and their translation into clinical management. Their usefulness in paediatric brain trauma remains unclear because of the lack of previous studies. An overview of the development in this area of monitoring is presented in the following sections.

2.6.1) Brain Tissue Oxygen Delivery and CO₂ Removal Monitoring

Over the past few years, the use of a fiberoptic sensor that is capable of measuring oxygen tension, CO₂ tension, pH, and temperature in the blood has gained considerable interests and has been adapted for use in adult brain tissue [218]. The multi-parameter sensor may be inserted into brain tissue together with the standard ventriculostomy catheter and a microdialysis probe through a triple lumen intracranial bolt. Evidence from animal studies suggested a potential clinical role for this multi-parameter sensor [218, 219], and several adult studies have been carried out to investigate its clinical usefulness in brain trauma [220, 221]. In one study of 20 brain trauma adults, those with severe injury had low brain oxygen tension in the first few hours following the initial insult [220]. A slow increase in brain oxygen

tension and a decline in the brain CO₂ tension to normal values was observed in those patient who recovered with a favourable outcome while a further decrease in brain oxygen tension to anaerobic levels was seen among those with bad outcome [220]. Furthermore, the brain CO₂ levels of 90 – 150 mmHg were seen consistently after brain death [220]. Continuous monitoring of the brain oxygen tension, CO₂ levels, pH and temperature may, therefore, provide additional information to aid the management of the comatose patients but require detail evaluations in larger cohorts. Transferability of its use in paediatric brain trauma is yet to be investigated.

2.6.2) Monitoring of Cerebral Blood Flow and Metabolisms in the Injured Brain

Although maintaining an adequate CPP is important as it provides a pressure gradient governing the cerebral blood flow, the ultimate determinants of the cerebral metabolism are cerebral blood flow (CBF) and the oxygen content in the blood. Monitoring of the ICP and CPP alone, therefore, offer no information on the brain's oxygen delivery and usage with some investigators of adult brain trauma considering this conventional practice 'un-physiological'. Cerebral blood flow targeted treatment may, therefore, be more 'physiological' and beneficial to neuronal recovery. At present, methods to measure cerebral blood flow such as Positron Emission Tomography (PET), Xenon clearance or Single Positron Emission Computed Tomography (SPECT) are too cumbersome for use at the bedside in the ICU. Jugular venous bulb oximetry and transcranial doppler ultrasound may be performed

in the ICU and may, therefore, provide a better bedside understanding of the state of the cerebral circulation and oxygen consumption.

2.6.2.1) Jugular Venous Bulb Oximetry

Arterio-jugular venous oxygen saturation differences (AVDO₂) allow assessments of the balance of the brain's metabolism and blood flow. Mixed venous oxygen saturation (SjvO₂) of blood leaving the brain can be measured from blood samples drawn from the jugular venous bulb situated at the base of the brain. This involves placement of a catheter directed upward in the internal jugular vein so that the tip of the catheter rests in the jugular venous bulb. Continuous SjvO₂ monitoring is possible by using a fiberoptic catheter.

In adults, the SjvO₂ is normally between 50 and 75 %. When the blood supply to the brain exceeds its demand for oxygen (hyperemia), an increase in SjvO₂ to more than 85% will occur. This may be the result of an increase in the cerebral blood flow i.e. cerebral hyperemia, or a relative hyperemia due to a decrease in cerebral metabolic rate associated with impending cell death/brain death. Up-to 55% of adults have been shown to develop hyperemia after brain trauma and there is a highly significant association between hyperemia and the occurrence of intracranial hypertension . As the cerebral blood flow responses to hyperventilation are generally preserved, brain trauma patient with hyperemia is more CO₂ reactive . Hyperventilation may be used to treat intracranial hypertension in these patients with the guidance of SjvO₂

monitoring to ensure cerebral ischemia does not occur as the brain's demand for oxygen changes during recovery.

Inadequate cerebral blood flow from an increased cerebral vascular resistance (CVR) will result in a reduction of $SjvO_2$ below 50% in the absence of a fall in arterial oxygen saturation (SaO_2). Elevation of cerebral vascular resistance occurs frequently after brain trauma and is made worse by hypocapnea. An increase in the cerebral metabolic rate would also cause a fall in the $SjvO_2$. Thus, in patients with persistent intracranial hypertension unresponsive to standard therapies and in the absence of evidence for cerebral hyperemia, barbiturates may be used to produce intermittent burst suppression to reduce the cerebral metabolic rate for oxygen ($CMRO_2$). The occurrence of jugular venous desaturation was demonstrated to be strongly associated with a poorer neurological outcome in adult brain trauma [222]. 90% of patients with multiple episodes of desaturation and 74% of those with one desaturation had a poorer neurological outcome compared to 55% of those with no episodes of desaturation [222].

The normal $SjvO_2$ values in infants and children remain unknown. Practical difficulties in siting the sizeable jugular bulb catheter in small children have limited its use in the paediatric population to selective cases of older children and adolescents with intracranial hypertension unresponsive to conventional first line therapies. None of our cohort had $SjvO_2$ measured during their ICU management. Further studies are needed to ascertain the clinical usefulness of $SjvO_2$ in childhood brain trauma and its relationship with outcome.

2.6.2.2) Transcranial Doppler Ultrasound (TCD)

Transcranial Doppler ultrasound (TCD) is a non-invasive method to assess flow velocities of the intracranial cerebral arteries and was first described in the early 1980s [223]. Flow velocities can be measured in the middle, anterior and posterior arteries by placing a probe in the temporal area just above the zygomatic arch. As the cross-sectional area of the arteries cannot be measured directly, flow cannot be measured from velocity, but the doppler shift measured is inversely proportional to the diameter of the vessel. Thus, provided all other factors remain constant, vasospasm will result in an increase in flow velocity.

Using transcranial doppler, middle cerebral artery spasm was identified in 40% of patients after traumatic brain injury and could begin as early as 48 hours post injury [224]. Maximal MCA spasm was found between 5 to 7 days after the initial trauma [224]. In another study of brain trauma adults, severe MCA spasm identified on the transcranial doppler was confirmed by angiography [225].

Daily measurements of the MCA velocity from 121 patients with varying severity of brain trauma showed an inverse correlation between the injury severity and the MCA velocity [226]. In addition, a significantly lower MCA velocity on admission was demonstrated among the brain injured patients when compared to those of normal controls [226]. High ICP and low cerebral blood flow were responsible for the low velocities in the intracranial circulation after brain trauma. Furthermore, an

admission MCA velocity of less than 28 cm/s correctly predicted 80% of the early deaths [226].

Currently, TCD remains a research tool and is not used in the clinical management of the brain trauma children. Furthermore, it only offers intermittent measurements of cerebral blood flow and its usefulness and accuracy in determining cerebral vasospasm is operator dependent. Its role in outcome prediction and in identifying brain trauma children at risks of developing vasospasm requires further investigations.

2.6.2.3) Microdialysis

As the composition of the cerebral interstitial fluid reflects the biochemistry of the neurons and glial cells in the brain, attempts have been made over the past decade to develop the use of cerebral microdialysis to monitor neurotransmitter release and energy metabolism in the animal and human brain. By mimicking the function of a blood capillary, the microdialysis catheter enable monitoring of the chemical changes in the interstitial fluid [227]. Because of a concentration gradient across the semi-permeable microdialysis membrane, chemical substances within the interstitial fluid diffuse across the dialysis membrane and into the perfusion fluid inside the catheter [227]. Samples are collected in microvials and brought to a bedside analyser as often as necessary [227].

The dialysate lactate, lactate/pyruvate ratio, glycerol, glutamate, glucose and adenosine levels have been investigated in the brain trauma adults [228-234]. The lactate/pyruvate ratio has been shown to be a better marker of ischaemia than lactate alone as an increase in lactate may be the result of hypoxia, ischaemia, or hyper-metabolism [228, 229]. But during ischaemia, neurons become dependent upon anaerobic metabolism to produce ATP from glucose. This will cause an increased in lactate production while pyruvate production decreases. As a result, there is an increase in the lactate/pyruvate ratio during ischaemia [228, 229]. Glycerol is an integral component of the cell membrane and has, therefore, been used as a marker of cell membrane damage [230, 231]. Although glutamate has been proposed as an indirect marker of cell damage, it is often hard to interpret the dialysate level as the neuronally released glutamate is mixed with the large metabolic pool of glutamate. A reduction in the dialysate glucose is often associated with a decrease in brain oxygen tension suggestive of reduced cerebral perfusion [232]. A mark increase in the cerebral interstitial fluid adenosine level has been shown to occur during jugular venous oxygen desaturations suggesting a potential role for adenosine during the periods of secondary insults after brain trauma [235].

As microdialysis samples from surrounding interstitial fluid, it, therefore, only offers monitoring of the local neurochemistry. The interpretation of microdialysis data depends upon the position of the catheter in relation to the existing pathology and its use in children requires investigations.

2.7) LIMITATIONS

This study has several limitations. Power calculation of sample size was not possible at the onset of the study because the main aim was to develop and validate a novel bi-dimensional index for quantification of pressure insult so that critical age-related CPP and thresholds may be defined. The original study proposal planned to recruit 140 brain trauma children over a two-and-a-half year period starting on 1 October 2000. This proposed sample size was based upon the estimated number of available patients calculated from the best available ICU admission figures in 1997. The prevalence of paediatric brain trauma had fallen sharply since the calculation of our sample size, which possibly reflected the success of the many accident preventative measures introduced. Re-organisation of the Scottish Paediatric ICU services had not increased the number of brain trauma admissions to the Edinburgh RHSC as expected. Despite successful recruitment of all available brain trauma patients into the study from all 3 sites, only half of the proposed number was enrolled by the end of the study period. The number ($n = 76$) was insufficient for meaningful statistical analyses. As a result, patients recruited from a prospective pilot study carried out in the late 1980s in the regional paediatric neuro-intensive care centre in Edinburgh [92] were included for analyses. This was only possible because their inclusion criteria and treatment protocol were the same as our current study and they did not differ statistically from our current cohort.

Despite inclusion of the pilot group to give our final cohort of 128 patients, not all patients had ICP monitoring. Once excluding patients aged less than 2 years and

those with ICP monitoring commencing after 24 hours of injury, the sample size reduced to 81, which is smaller than our original anticipated number. Although the sample size was smaller than intended, we were able to develop and validate our novel index for pressure insult quantification.

Majority of our patients had GOS 4 and 5 (i.e. independent outcome), with very few patients had severe disability or a fatal outcome. This uneven spread of outcome made it necessary to assess the influence of physiological derangement and CPP insult on outcome using dichotomies which included patients with severe disabilities (GOS 3) and fatal outcome in the same category. Despite this limitation, we have demonstrated that CPP insult predicted death and those with poor outcome (GOS 1-3).

Our study included children of different ages up-to 16 years. Like other paediatric brain trauma cohort, patients are not evenly distributed across the different age ranges. Because of the diverse and uneven patient distribution across age ranges, our cohort was divided into 3 age bands for analyses. As a result, we have only managed to propose critical CPP thresholds for 3 different age bands. A much larger cohort in each year of age with physiological recordings in minute resolutions will be required to define critical CPP thresholds for each age group, but our current study has provided the necessary information to calculate sample size for future studies.

CPP data was calculated from continuous recordings of arterial blood pressure and intracranial pressure. Variations in the positions of the pressure transducer across

recruitment units or under-damping of fluid-filled catheter-transducer system may influence the accuracy of the recorded arterial blood pressure value with the potential to introduce error into data analysis. This was minimised in our study because the pressure transducers were zeroed to the external auditory meatus in all recruitment sites.

Our study did not examine cerebral autoregulation when it is an important determinant of brain trauma outcome. We chose not to include cerebral autoregulation assessment in our study because there is no gold standard for cerebral autoregulation assessment and continuous assessment of cerebral autoregulation is not yet available with all currently available methods only offering intermittent evaluation of cerebral autoregulation. Despite this limitation, CPP insult quantification was achieved and its high outcome predictive value was demonstrated.

Our novel cumulative pressure time index (PTI) is a powerful tool for summarising the total burden of CPP or ICP insult bi-dimensionally. Because it is calculated post hoc, it is a research tool and is, therefore, not useful for clinical management of brain trauma patients at the bedside. However, its development and validation has added significant values to the brain trauma literature by providing a novel method to quantify the total burden of pressure insult. In addition, it enables definition of 3 age-related critical CPP thresholds which has not been possible previously with the various existing methods to quantify pressure derangement in single dimension. Furthermore, with PTI, we may now investigate whether ICP/CPP values measured

in minute resolutions offer more accurate insult summary and outcome prediction than may be achieved using pressure recordings at hourly intervals.

2.8) CONCLUSIONS

A novel index combining the degree and duration of physiological derangement has been developed. This is the first method to include more than one dimension in the analyses of ICP and CPP data from brain trauma patients. Using pre-defined age-specific theoretical physiological threshold values for ICP and CPP, we have demonstrated that the index is robust and relates extremely well to both morbidity and independent outcome. This novel index has opened up future brain trauma research opportunities in critical care medicine particularly in relation to (i) determination of the total burden of secondary physiological insults, (ii) definition of critical ICP and CPP physiological thresholds, (iii) validation of current treatments, and (iv) development of novel therapies.

Our pre-determined age-related physiological based theoretical CPP thresholds have been proven to be the critical minimum CPP thresholds since altering these original threshold levels failed to improve the predictive value of the PTIc on outcome. We believe that for children age-related critical physiological CPP thresholds are in fact identical to the insult thresholds.

CHAPTER 3: VARIABILITY AND INTER-RELATIONSHIPS OF ICP, CPP, AND MAP SIGNALS AND THEIR RELATIONSHIPS WITH OUTCOME AFTER CHILDHOOD TBI

3.1) INTRODUCTION

Prompt detections and corrections of secondary physiological derangement after brain trauma are the basis of modern neuro-surgical and neuro-intensive care and may improve outcome. The success of modern neuro-intensive care, therefore, relies heavily upon simultaneous monitoring of multiple biosignals continuously. Rapid development in computing technology over the past three decades means that it is now the expected norm to use computerised monitoring in intensive care settings. Large quantity of raw data is generated as a result which could be analysed in detail off-line at a later stage and the findings of these analyses, in turn, contribute towards improving neuro-intensive care treatments.

Despite the endless research interests in the pressure signals of intracranial and cerebral perfusion pressure and the vast quantity of literature generated, relatively few described their variability after traumatic brain injury. These early studies describing variability of pressure signals used data from paper recordings [160-162], but were hindered by the many technical difficulties. These difficulties included lengthy traces, chart speed variations, and a lack of objectivity in the quantification and analysis of the data. Despite the already established norm to employ computerised monitoring in modern intensive care, there have been little attempts to

re-examine the relationships between the variability of pressure signals and outcome after brain trauma.

3.2) AIMS

This chapter aims to test the hypothesis that the variability of the pressure signals over time after childhood brain trauma may be important in relation to outcome.

3.3) PATIENTS AND METHODS

3.3.1) Design of the Study

A prospective observational study of critically ill brain injured children with global outcome assessment at 6 and 12 months post injury was conducted to view time-series data (artefact-excluded) over extended time periods, to investigate the significance of the observed variability and to identify other practical but simple visual displays available at the bedside.

3.3.2) Patients

Forty-three children admitted to the Regional Head Injury Unit in the South-East of Scotland between 1 January 1989 to 16 June 1996 were included in the study if the following criteria were fulfilled:

- a) There was a post-resuscitation pre-intubation Glasgow Coma Score [28] sumscore (GCSs) of 12 or less. The Pediatric Glasgow Coma Score [236] was used for children aged 5 years and under;
- b) The GCSs was >12 in association with an Injury Severity Score (ISS) [237] of 16 or more;
- c) There were clinical indications for monitoring the patients in the Intensive Care Unit and continuous ICP monitoring was available;
- d) There was a Computerized Data Collection (CDC) system available for use within 24 hours of injury.

Children were excluded from the study if they had previously sustained a brain injury. Ethical approval for this study was granted through the local ethics committee as part of a larger study of secondary insults, and assent were obtained from parent or guardian prior to entry into the study. These children were managed using a standardised TBI management guideline which had been described previously in Chapter 2.

3.3.3) Data Collections

3.3.3.1) Demographic Data

Demographic and clinical data collection had been described in details previously in Chapter 2 and included the cause and nature of injury, age, Glasgow Coma Score on

admission and, after acute non-surgical resuscitation, pupil responses, X-ray and Computerised Tomography (CT), operative and treatment details.

3.3.3.2) Physiological Data

Details on the acquisition of the physiological data could be found in Chapter 2. Data were collected every minute until clinical monitoring ceased. They were then manually validated using the Edinburgh Browser® software and any artefact or unreliable data were excluded. For this study, the pressure signals (ICP, MAP and CPP) data used were restricted to the duration of the ICP monitoring, and from quantitative statistics the mean, range and standard deviations were calculated, using all valid data for each of the parameters.

In addition the data were divided into epochs of 48-hour, taken from the time of injury, and the same descriptors recorded for each epoch. Up to 6 epochs were recorded for each patient. The first epoch, taken from the time of injury always had less than 48 hours of data, as this epoch necessarily included the transfer time, admission time and “set up” time. Similarly, the last epoch for each child could be less than 48 hours, as this depended on a clinical decision as to when the ICP monitor was removed.

For each patient time series plots were prepared for each epoch, where the 3 pressure signals were displayed using the same vertical axis of mmHg. Each 48-hour epoch was then assigned a ‘pattern type’, by consensus among 3 researchers.

3.3.3.3) Outcome Data

Global outcome assessment was performed at 6 and 12 months post injury and the details of the questionnaire used could be found in Chapter 2. Outcome was dichotomised into ‘recovered’, i.e. good recovery and moderate disability (GOS 4-5) and ‘not recovered’ i.e. severe disability, vegetative state or death (GOS 1-3).

3.3.4) Analyses

Microsoft® Excel 97 and SPSS® for Windows (Release 9.0.0) packages were used to analyse the data, employing Mann-Whitney U, Kruskal-Wallis and Chi Square tests, and the significance level of $p < 0.01$ to ensure non-chance relationships.

3.4) RESULTS

3.4.1) Demographic Results

All 43 children had intracranial pressure (ICP) monitoring, with complete data for arterial pressure and cerebral perfusion pressure (among other parameters). The demographic features of the group, including age, sex, and outcome at 12 months post-injury, are summarised in Table 3.1.

Table 3.1:

Demographic data for 43 children with ICP monitoring				
Sex	Males			27
	Females			16
Cause	Vehicle / bicycle			13
	Pedestrian			22
	Falls			4
	Other			4
Grade	Severe (GCS \leq 8)			32
	Moderate (GCS 9 – 12)			9
	Minor (GCS 13 – 15 with ISS \geq 16)			2
Pupils	Some reaction			36
	No reaction			5
	Not testable			2
CT	Focal			24
	Diffuse			15
	Less severe injuries			4
Outcome at 12 months by Grade on admission				
	Severe	Moderate	Minor	Total
GOS 1	8	1	-	9
GOS 2	-	-	-	-
GOS 3	2	-	-	2
GOS 4	6	3	-	9
GOS 5	16	5	2	23
Total	32	9	2	43

Monitoring times ranged from 97 – 17 778 minutes (mean = 6319; median = 3446), a total number of 221 291 data points were, therefore, available for analysis. There were a total of 104 epochs (of 48-hour) available for analysis. Epochs 1 through 6 had data from 42, 28, 19, 10, 4 and 1 patient respectively. The mean number of epochs per patient was 2.4. In one case, computer data were lost during the first epoch and therefore excluded from the analysis.

3.4.2) Variability

Table 3.2 shows results for the total duration of acute monitoring i.e. ICP monitoring time and the first 3 consecutive epochs (i.e. admission to 48 hr post injury [epoch 1]; 48-96 hrs [epoch 2] & 96-144 hrs post injury [epoch 3]). There were insufficient data for analysis of variability in epochs 4 to 6.

Table 3.2:

		1ST EPOCH INJURY TO 48 HOURS		p VALUES
Test	(n)	Alive vs. Dead Mann Whitney U (42)	Recovered vs. Not recovered Mann Whitney U (42)	
<i>ICP</i>	Mean	<0.001*		0.001*
	S.D.	0.011		0.008*
<i>MAP</i>	Mean	0.053		0.021
	S.D.	0.193		0.191
<i>CPP</i>	Mean	0.049		0.380
	S.D.	0.716		0.275
		2ND EPOCH 48 – 96 HOURS POST INJURY		p VALUES
Test	(n)	Alive vs. Dead Mann Whitney U (28)	Recovered vs. Not recovered Mann Whitney U (28)	
<i>ICP</i>	Mean	<0.001*		0.006*
	S.D.	0.365		1.000
<i>MAP</i>	Mean	0.806		0.709
	S.D.	0.682		0.784
<i>CPP</i>	Mean	<0.001*		0.006*
	S.D.	0.978		0.901
		3RD EPOCH 96 – 144 HOURS POST INJURY		p VALUES
Test	(n)	Alive vs. Dead Mann Whitney U (17)	Recovered vs. Not recovered Mann Whitney U (17)	
<i>ICP</i>	Mean	0.006*		0.279
	S.D.	0.953		0.879
<i>MAP</i>	Mean	0.300		0.442
	S.D.	0.953		0.721
<i>CPP</i>	Mean	0.244		0.646
	S.D.	0.859		0.959
		TOTAL DURATION OF ICP MONITORING		p VALUES
Test	(n)	Alive vs. Dead Mann Whitney U (43)	Recovered vs. Not recovered Mann Whitney U (43)	
<i>ICP</i>	Mean	<0.001*		<0.001*
	S.D.	<0.001*		<0.001*
<i>MAP</i>	Mean	0.067		0.036
	S.D.	0.003*		0.017
<i>CPP</i>	Mean	<0.001*		0.003
	S.D.	0.005		0.021
* Sig at p <0.01				

3.4.2.1) Total Duration

The *variability* of ICP, MAP and CPP (as measured by the standard deviation) over the whole time that the patients had an ICP monitor in place, was significantly different between those who died and survived. Variability of ICP significantly related to outcome (GOS at 12 months post-injury), and was also significantly greater in those who did not recover compared to those who recovered.

Mean ICP and mean CPP for total duration of ICP monitoring were significantly related to outcome in the 3 outcome tests.

3.4.2.2) 1st Epoch (First 48 Hours from the Time of Injury)

The *variability* of ICP was significantly greater during the first 48 hours of injury in those who recovered compared to not recovered. However, the variability of MAP and CPP during the first epoch did not appear to be related to outcome in this study.

3.4.2.3) 2nd Epoch (48 – 96 Hours from the Time of Injury)

By this time frame, the variability of neither ICP, MAP nor CPP were shown to be significantly related to outcome, however, all the mean values of mean ICP and CPP were related to outcome at the 1% level.

3.4.2.4) 3rd Epoch (96 – 144 Hours from the Time of Injury)

During this epoch, there was no relationship between the variability of any test signal and outcome and only the mean value of mean ICP was related to survival ($p=0.006$).

3.4.3) Patterns

When 3 variables are displayed on the same graph using a common vertical axis, there are necessarily 6 permutations, in terms of the order of magnitude in which the variables could theoretically be presented. However, because of the mathematical relationship, $CPP = MAP - ICP$, only 3 physiological ordered combinations are possible.

3.4.3.1) Descriptions of Patterns (see Figure 3.1)

Type I – where MAP was greater than CPP, which was greater than ICP (this would be considered the ‘normal’ and desirable relationship between the variables.

Type II – where MAP was greater than ICP, which was greater than CPP. The ICP is so high that there is insufficient compensation in systemic pressure to maintain adequate CPP.

Type III – where ICP was greater than MAP, which was greater than CPP. With grossly elevated ICP there is total failure of compensation by systemic pressure and CPP is absent.

Type II / I Cross-over – where MAP remains greatest, but CPP changes from being lower than ICP to greater than ICP (i.e. an improving pattern, and is actually a changing pattern from Type II to Type I).

Type I / II Cross-over – where MAP was greatest, but CPP changes from being greater than ICP to less than ICP (i.e. a deteriorating pattern, or changing from Type I to Type II).

Type I / II / III Cross-over – generally where MAP remains the highest of the three variables, but ICP and CPP repeatedly reverse their relative positions, i.e. fluctuating between type I and Type II. The multiple changes could also be a pattern that fluctuates between Type II and Type III.

Figure 3.1a

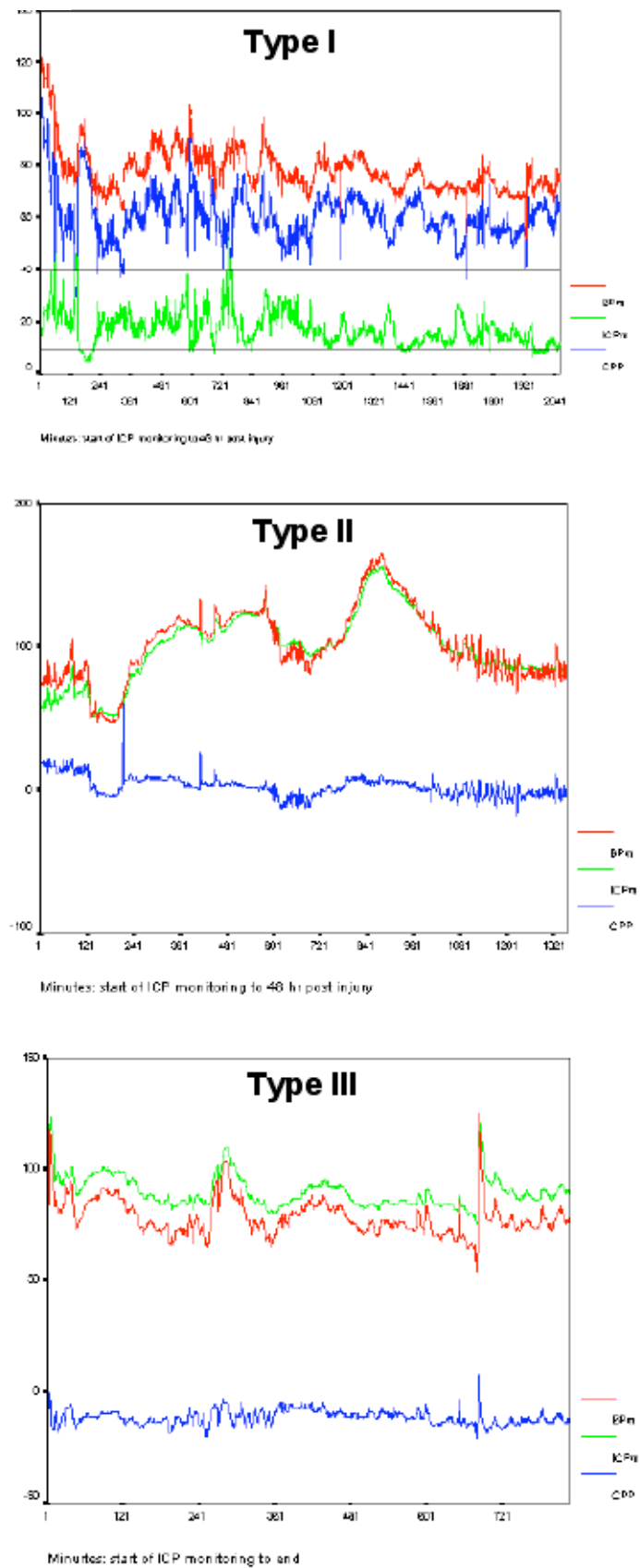
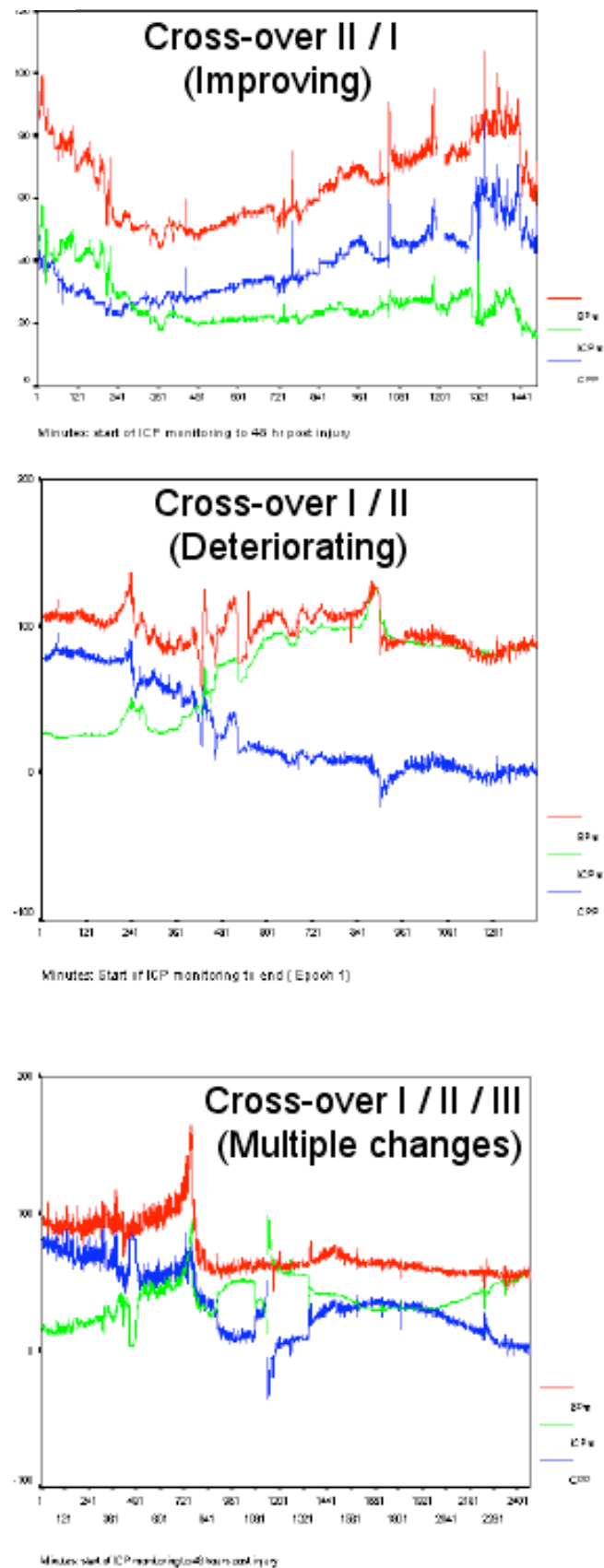


Figure 3.1b



3.4.3.2) Patterns in Relation to Outcome Scores

104 epochs (of 48 hours) were identified from the data of 43 patients. We found no instances of Type III reverting to Type I. There was only 1 epoch (in 1 patient) with an improving cross-over pattern (Type II / I), and this epoch has been included with the Type I group for analysis. Overall 93 (89.4%) epochs displayed Type I pattern. GOS scores at 12 months were highly significant in relation to pattern type (Chi Sq. test $p = <0.0001$, Fisher's exact Test).

3.4.3.3) Patterns in Relation to Fatal Cases

Nine children died, presenting a total of 20 epochs. 9 of these epochs were described as Type I. Four patients who died *only* displayed cross-over patterns and at no time had a Type I. Of the remaining 5, 4 had episodes of Type I (9 epochs) that subsequently changed to Type II, III or Cross-over. No patient survived if they displayed *at any time epoch* any of the following patterns: Type II, Type III, Cross-over Types I / II or Type I / II / III.

The one patient, who did not survive but displayed just one epoch of Type I pattern, eventually died after all monitoring was withdrawn, some 2 days later. Unfortunately data were lost for all the subsequent epochs.

3.5) DISCUSSIONS

Modern computerised monitors allow multiple clinical parameters to be displayed simultaneously providing trends in the clinical conditions. Their ultimate aim is to alert clinicians to any significant changes in the patient's condition so that appropriate treatment may be instigated. However, with the rapid advances in monitoring techniques, clinicians face the danger of having data overload while interpreting and responding to a great number of increasingly complex clinical parameters. There is, therefore, an urgent need to summarise these data for meaningful interpretation.

Prior to the development of computerised data storage and usage of the more elaborate analyses such as artificial intelligence techniques [238] and decision-tree induction [239], the only way to obtain a permanent copy of the physiological monitoring and to condense the data into a manageable format for interpretation was by manually transcribing data displayed on the ICU monitors onto specially designed charts at specific time points, e.g. hourly creating a snapshot in time. The advantage of this method included the ability to view trends over longer time periods than as seen on the monitor screen, but it was disadvantaged by excluding almost all the data made available with the new continuous monitoring technology. Furthermore, it utilized valuable specialist ICU nursing time.

Most modern ICP research still uses these hourly data transcribed by nurses onto observation charts. Some researchers have used specific time points such as overall

time taken from commencement of monitoring [72, 91] which gives no indication of how long between injury and arrival in ICU. Others have truncated the data to particular time durations, for examples, 24, 48 and 72 hours from the point of impact [240], or averaged the data over 24 hours [71] or 12 hours periods [241]. Because on-line data produces lengthy files we conducted our analysis in 48-hour epochs as well as for the total time.

Each of these methods has disadvantages as the length of ICP monitoring continues to be determined by clinical conditions. Monitoring may be removed early in those who recovery quickly or after physiological stability is achieved. Cortbus [199], describing the "stages" in the pathophysiology of TBI, found that the majority of hypotensive insults occurred with the first 24 hours, and ICP derangements clustered around days 5 and 6, although insults were detected up to 12 days. Therefore, by choosing 48 hours as our standard epoch, we compromised between a duration long enough to show variations (the cross-over patterns generally take more than 12 hours to develop), but not so long that there were overwhelming amounts of data to handle. Epochs were well represented with patient deaths occurring in each epoch, and withdrawal of ICP in each epoch.

We have not recorded the presence of abnormal ICP wave forms, such as plateau and B waves, which may influence the standard deviation of these relatively short-duration waves forms but their effect would be relatively minimal when compared to all ICP data points over a 48-hour epoch. Additionally, pre-morbid data i.e. any data

that were recorded within the last 4 hours before death was excluded in this analysis as is common practice, since pre-morbid data by definition can only predict death.

Many methods of analysing pressure signal data have been described previously, including using the maximum [69, 76] or mean ICP [71]; durations of secondary insults [72]; mean CPP [91, 242]; times above and below specific thresholds [243]; “mean average”, and “mean worst” ICP or CPP (in 12-hour epochs) [241] and using rolling two-minute averages [244], and each method highlighted a relationship with outcome. However, each paper described a different methodology to summarise the data, suggesting that there is no universally accepted standard analysis. More elaborate analysis has included artificial intelligence techniques [238] and decision-tree induction [239]. Our method described here adds another method to the literature, but is simple to perform and aids the understanding the plethora of data presented at the bedside [245].

3.5.1) Variability

Similar to findings of previous ICP studies in the brain trauma literature, the mean values of ICP and CPP in this study were highly significant in most of the epochs in relation to outcome [245]. However, the *standard deviation of ICP* was the only significant predictor (at $\alpha = 0.01$) of non-recovery, and only then in the 1st 48-hours from injury [245].

Signorini and colleagues examined "insults added to injury" at 24, 48 and 72 hours post injury in a population of head injured adults [240]. Various summary measures were examined including the presence or absence of any grade of insult, the total time (and proportion of time) spent in insult of grade 1 or worse, and a weighted sum or proportion of monitoring time at specific grades of insult [240]. Importantly, they concluded that raised ICP *however summarised* was independently predictive of mortality at each of these times [240]. Our findings add another simple summary measure to the list mentioned previously, at a clinically useful time (48 hours after injury) which is predictive of morbidity [245]. No other test of variability in any other epoch was significant at this stringent 1% level.

A different picture was seen however, when considering the 3 pressure signals over the total duration of 'acute' monitoring time (i.e. time that ICP monitoring was in situ). In this case, the variability of all three traces was highly significant in those who died, and the variability of ICP was significantly related to outcome in the other two comparisons, reflecting the cumulated changes over time [245]. *Practically*, however, the standard deviation (i.e. the variability) over the *whole* trace (if more than 48 hours) is not a useful predictor during the acute care of these children, as the variability only becomes significant in retrospect (when it is already evident that the child has survived, or has died). It may be useful for counselling grieving parents and families because the displayed trends may summarise information in such a way that may be readily understood by individuals without medical knowledge.

Two other groups of investigators (Marmarou from the Traumatic Coma Data Bank group [246] and Gaab [162]) have used standard deviation as an index of ICP variability. Marmarou used ICP ‘variance’ (standard deviation squared), which was considered to be an indirect measure of the pressure stability, but found that it was only poorly related to outcome [246]. This may be because only ‘end hour’ readings were used which were considered to be an average estimate of the whole hour, giving only 24 data points per day, compared to our 1440 per day for each variable. Clearly, the fewer the data points used, the less variability there is to measure. Gaab [162] studied a group of 16 head injury patients (including both adults and children) out of a total group of 72 with ICP monitoring ranging from 24-280 hours duration. While these study durations are comparable to those in ours, he found the mean ICP ranged from 20-50 mmHg, and the standard deviation ranged from 4-21 mmHg, both parameters showed less variability than found in our study of 43 children (mean range 2.56-99.36 mmHg; S.D. range 1.53-32.21 mmHg) after artefactual data were removed.

3.5.2) Pattern Types

By creating time-series plots using the data of the three pressure signals (ICP, MAP, and CPP) along the same vertical axis, the pattern of the plot may be readily categorized using ‘the order of magnitude’. We demonstrated that the pattern type displayed were predictive of survival or death since (i) all patients who exhibited patterns of Type II, Type III, multiple cross-over (type I/II/III) or simple cross-over (Type I/II) died; and (ii) all patients with Type II / I (changing from a ‘poor’ to

‘better’ type of pattern) survived, indicating a response to treatment [245]. Furthermore, the pattern type identified has unanimous agreement among different researchers indicating the classification was robust and easily applicable in clinical practice. This could, therefore, be a simple but yet useful addition to the traditional nurse transcribed observation charts, where generally numerical values for ICP and CPP are recorded on a separate section of the chart, thus obscuring the information.

It was noted that it would be possible to further subdivide *Type I* patterns depending on the amount of variability seen in the ICP trace, for example by identifying those with only 10 mmHg within-trace variation, or 20, 30, 40 or >40 mmHg within-trace variability. We were unable to relate these sub-group patterns to outcome in this sample group of patients as examples were found in all outcome groups. Amalgamating Type I sub-categories into ≤ 30 mmHg or > 30 mmHg of variability revealed 14% (7/43) of those with less variability had a poor outcome while 21% (9/43) of those with greater variability of Type I pattern had a poor outcome. While not statistically significant in this small patient group, the method may be useful in larger groups with Type I patterns, to identify a threshold variability which predicts a less desirable pattern type.

By viewing these patterns over 48-hour time frame, it appeared in some cases as if there was a rhythmical character to the slow wave forms seen. The evident rhythmicity made us consider if there were some other underlying wave forms, but on close analysis these were 4 hour treatment-related events and not an inherent hydro- or haemo- dynamic pulse.

3.6) CONCLUSIONS

The standard deviation (or variability) of ICP signals frequently recorded during ICU care of paediatric TBI patients only gives useful predictive information about outcome during the first 48 hours after injury.

The “time series patterns” generated from traces of three variables (ICP, MAP and CPP) when plotted on the same vertical axis, show clearly which patients are likely to survive or die, and these are useful throughout the whole acute monitoring time. If these values were plotted on standard ICU nursing charts, it could form a simple but useful visual adjunct to other methods already available in the management of TBI in the Intensive Care Unit.

CHAPTER 4: MODULATING EFFECT OF APOLIPOPROTEIN E POLYMORPHISMS ON SECONDARY INSULT AND OUTCOME AFTER CHILDHOOD BRAIN TRAUMA

4.1) BACKGROUND

Recovery from childhood brain trauma remains diverse and sometimes appears out of proportion to the severity of primary brain injury and the amount of secondary insult experienced. This suggests that there may be other influences, for example genetic make-up, which may be an additional determinant of outcome after brain trauma.

Potential genetic influence on outcome after brain injury has increasingly been investigated over the past decade. The Apolipoprotein E (APO E) gene has secured a central role in this research area because this 5083-nucleotides gene is polymorphic in human with 3 allelic forms (e2, e3, and e4) [247] and its gene product, apolipoprotein E (apo E), is a major lipoprotein involved in lipid transport and metabolism within the central nervous system (CNS) [144], which is thought to be particularly important for the repair process after CNS damage.

The influence of the APO E polymorphisms on neurological recovery may be related to the presence of the e4 allele because its possession has been associated with increased risk of developing Alzheimer's Disease [248, 249] and poorer outcome following various acute brain injury [141, 142, 250]. Furthermore, the number of e4

allele present appears to govern its allelic effect on the risk of developing Alzheimer's Disease where the e4 homozygous conferred the highest risk, while e4 heterozygotic subjects had an intermediate risk, and individuals without the e4 allele possessed the least risk [249]. Teasdale's group speculated a similar gene dose effect on brain trauma recovery since all their e4 homozygotic patients (n=4) suffered severe disability after brain trauma, 8 (31%) of their 26 heterozygous e4 recovered with a poor outcome, but only 11 (17%) of the 63 patients without the e4 allele had an unfavourable outcome [141]. However, no other clinical brain injury study in adults or children has confirmed this potential gene dose effect.

Animal studies suggest that the absence of the e4 allele may affect recovery after brain injury. Following closed head injury, the APOE e3 transgenic mice recovered more rapidly than the controls, APOE deficient, and APOE e4 transgenic mice in both neurological and histological analysis [251]. Furthermore, adult transgenic mice expressing the human APOE e4 allele are shown to be more susceptible to fatality from closed head injury than those expressing the e3 allele [251]. Buttini and colleagues found that hemizygous and homozygous transgenic mice with human APOE e3 allele were protected against age-related and excitotoxin-induced neurodegeneration (by injection with kainic acid which is a glutamate antagonist) while those expressing APOE e4 were not [252]. Apolipoprotein E e3/e4 bigenic mice were as susceptible to neurodegeneration as APOE e4 singly-transgenic mice [252]. Although the translation and transferability of these findings into clinical setting remain unclear, these animal data, in addition to confirming the association

between APO E e4 allele and poorer neurological recovery, has suggested that the absence of the e4 allele may offer neuro-protection.

Precisely how APO E polymorphisms affect neurological recovery in human remains unknown. Postulated mechanisms have originated mostly from animal or in-vitro investigations and include potential allelic-related differential influence on the host's response to oxidative stress [253] or excitotoxicity [254, 255], and the rate of conversion of the neuroprotective β amyloid precursor protein (β APP) to the neurotoxic β amyloid (β A4) [256] but the clinical relevance of these mechanisms remains unclear. Despite the vast literature supporting the predictive value of CPP insult on brain trauma outcome in adults and children, the relationships between APO E genotypes and CPP insult following brain trauma has not been investigated previously.

4.2) AIMS

This chapter aims to test the hypothesis that Apolipoprotein E genetic polymorphisms differentially affect the burden of CPP insult and outcome after childhood brain trauma.

4.3) PATIENTS AND METHODS

4.3.1) Design of the Study

A prospective case-controlled study was carried out over a two and a half year period (1 April 2001 to 30 September 2003). Local ethics and hospital research management committees approved the study. Parental consent was obtained for inclusion in the study.

4.3.2) Patients

Brain injured children admitted to the ICU at the Edinburgh RHSC and Newcastle General Hospital during the study period and had already been enrolled into the secondary physiological derangement study were eligible for inclusion in this study. Sixty-five of the 70 eligible children were recruited successfully into the study.

4.3.3) Controls

160 healthy active age and sex matched children were recruited from the Accident & Emergency Department at the Edinburgh RHSC as control subjects. They all attended the A&E Department with minor injuries and had no previous history of head injury.

4.3.4) Sample & Data Collections

All participants had buccal smears collected for DNA extractions and subsequent Apolipoprotein E genotyping. Demographic and clinical details of the brain trauma cohort were collected prospectively as described previously in Chapter 2. In addition, their routinely monitored physiological parameters in minute resolutions were prospectively downloaded from the PICU bedside monitors for detection of age-specific physiological derangements as described previously in Chapter 2 using the Edinburgh Browser programme [92]. The total burden of CPP insult was quantified using the cumulative pressure time index for CPP (PTIc) [204] in patients with ICP monitoring. Forty-five children had continuous ICP monitoring but 7 were excluded from this subgroup analyses because significant amounts of ICP data were lost during the first 24 hours post injury from computer downtime.

4.3.5) Outcome Assessment

Conscious level was determined at PICU discharge which equated to the end of neuro-intensive care when the brain injured children no longer required airway protection, ventilatory and circulatory support. The modified Glasgow Coma Scale (GCS) [236] was used for this assessment. In addition, global outcome was assessed at 6 months post injury using the modified Glasgow Outcome Score (GOS).

4.3.6) Laboratory Methodology

I collected all buccal smear samples, developed and conducted all DNA extraction and APO E genotyping.

4.3.6.1) APO E Genotyping

4.3.6.1.1) DNA Extraction From One Buccal Brush

The PUREGENE DNA Buccal Smear Isolation Kit™ (produced by Gentra Systems) was used for collection of buccal smear and DNA extraction.

4.3.6.1.1.1) Buccal Smear Sample Collection

A sterile nylon bristle cytology brush (provided as part of the PUREGENE DNA Buccal Cell Isolation Kit™) was used to scrape the inside of the mouth (cheek) for 10 strokes. The brush was then dipped up and down 10 times in a 1.5 ml microfuge tube containing 300 µl of Cell Lysis Solution. When the brush was removed from the Cell Lysis Solution, care was taken to ensure minimal amount of solution was lost. Buccal smear was confirmed to be floating in the Cell Lysis Solution under direct vision. The sample was then stored at room temperature until sufficient number of samples (at least 10 but no more than 20 samples) had been collected to allow for batch DNA extraction. The buccal cells were stable in the Cell Lysis Solution at room temperature for 18 months.

4.3.6.1.1.2) Cell Lysis

1.5 µl of Proteinase K Solution (20 µg/ml) was added to each sample. Each microfuge tube was then inverted 25 times before overnight incubation at 55⁰C. This allowed for maximal cell lysis to achieve maximum DNA yield. The samples were then taken out of the oven and cooled for 35 minutes in room temperature before proceeding to RNase treatment.

4.3.6.1.1.3) RNase Treatment

1.5 µl of RNase A Solution was added to the cell lysate which was then inverted 25 times. The cell lysate was incubated at 37⁰C for 60 minutes. The samples were then cooled to room temperature which usually took 35 minutes before proceeding to protein precipitation. This ensured maximum final DNA yield and minimised the risk of protein contamination.

4.3.6.1.1.4) Protein Precipitation

100 µl of Protein Precipitation Solution was added to each of the cell lysate. Each microfuge tube was then vortexed at high speed for 20 seconds to ensure uniform mixing of the Protein Precipitation Solution with the cell lysate. The mixed cell lysate was placed immediately in an ice bath and remained in the ice bath for 5 minutes. The microfuge tubes were then centrifuged at 12 000 x g for 3 minutes.

The precipitated proteins should form a loose white pellet after the first centrifugation.

To ensure complete protein precipitation, the following steps were repeated: (i) high speed vortex (20 seconds), (ii) ice bath incubation (5 minutes), and (iii) centrifugation at 12 000 x g (3 minutes) until the formation of a tight white pellet. This was usually achieved within 2 – 4 cycles.

4.3.6.1.1.5) DNA Precipitation

The supernatant containing the DNA was poured into a clean 1.5 ml microfuge tube containing 300 µl 100% Isopropanol (2-Propanol, molecular biology grade) and 0.5 µl Gentra Glycogen Solution (20 µg/ml) leaving behind the precipitated protein pellet. The solution and supernatant was gently mixed by inverting the microfuge tube 50 times. The microfuge tubes were left in room temperature for 10 minutes before centrifugation at 12 000 x g for 5 minutes. The extracted DNA was usually visible as a small white pellet.

The supernatant was poured off and the tubes were drained on a clean absorbent paper. 300 µl of 70% Ethanol was then added to each tube. The tubes were then gently inverted 10 times to wash the DNA pellet. The tubes were then centrifuged at 12 000 x g for 1 minute. The ethanol was carefully poured off while ensuring the pellet remained in the tube. Each of the tubes was then inverted and drained on a

clean absorbent paper and allowed to air dry for 15 minutes. The DNA pellet was then hydrated within 20 minutes.

4.3.6.1.1.6) DNA Hydration

20 µl of the DNA Hydration Solution was added to each tube containing the DNA pellet. Immediately upon adding the DNA hydration solution, each tube was tapped 10 times and then incubated at 65⁰C for 1 hour. The tubes were left in room temperature overnight with periodic tapping of each tube to aid dispersal the DNA. The DNA was then stored in –70⁰C until genotyping.

4.3.6.1.2) Polymerase Chain Reaction (PCR)

Stored DNA samples were thawed in room temperature, which usually took 30 minutes, and the samples were vortexed briefly (for up to 8 seconds) at 12 000 x g. 5 µl of the DNA was then added to a 0.6 ml microfuge tube containing 45 µl of the PCR mastermix made up with the following components:

- 24.55 µl of deionised water
- 5 µl of x10 PCR buffer (MgCl₂ free)
- 8 µl of dNTPs
- 1.8 µl of MgCl₂
- 5 µl of betaine
- 0.2 µl of R₃
- 0.2 µl of L₃

- 0.25 µl of Taq polymerase

4.3.6.1.2.1) PCR Mastermix Components

Components of the PCR mastermix are detailed below:

- x10 PCR buffer (MgCl₂ free) and MgCl₂ were available as part of the Taq Polymerase Pack produced by Invitrogen (Cat. Number 18038-067).
- dNTPs used were produced by Amersham Pharmacia (Cat. Number 27-2035-02) which consisted of the following components: (i) Deoxyadenosine triphosphate (dATP) (100mM), (ii) Deoxycytosine triphosphate (dCTP) (100 mM), (iii) Deoxyguanine triphosphate (dGTP) (100 mM), and (iv) Deoxythymidine triphosphate (dTTP) (100 mM). The dNTPs was reconstituted as follows: 12.5 µl of dATP, dCTP, dCTP and dTTP were thoroughly mixed with 950 µl of de-ionised distilled water (molecular biology grade). The re-constituted dNTPs were divided into 100 µl aliquots for storage at – 20⁰C until use.
- Betaine (1.7mM) which assisted the breakage of G-C bonds during PCR was available as dry powder through Sigma (Cat. Number B-2629). 5.86 g of betaine powder was dissolved in 10 ml of de-ionised distilled water (molecular biology grade). The betaine solution was aliquot into clean eppendorfs and stored at – 20⁰C until use.
- Two 100pM/µl custom oligo primers (R₃ and L₃) were available through MWG-Biotech. The sequences of these primers are shown as follows:
R₃ = 5' - ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A – 3'
L₃ = 5' – TCC AAG GAG CTG CAG GCG GCG CA – 3'

The primers were re-constituted as per instructions on the synthesis report and stored at -20°C until use.

4.3.6.1.2.2) PCR Programme

PCR was carried out in PCR machines with the following PCR programme settings:

1. Pre-heat the lid for 2 minutes at 105°C
2. 95°C for 2 minutes (Prerun)
3. 94°C for 30 seconds (Denaturation)
4. 62°C for 30 seconds (Annealing)
5. 72°C for 1 minutes (Extension)
6. Repeat steps 2 – 4 for 35 cycles
7. 72°C for 5 minutes (Delay)

4.3.6.1.2.3) Detection of PCR Product Using Gel Electrophoresis

3% agarose gels were used to detect PCR products. 2 μl of the gel loading buffer was mixed with 10 μl of the PCR product before loading into the well. 5 μl of the 1 Kb ladder marker was used. After electrophoresis at 120V for 30 minutes, the gel was then read under UV light.

4.3.6.1.2.3.1) 3% Agarose Gel

The following steps were carried out to make one 3% agarose gel tray with 32 wells (maximum 104 wells).

1. 100 ml 1xTBE was added to a flask.
2. 3g of agarose powder was added slowly to the TBE while shaking the flask gently to avoid forming clumps.
3. A stir bar was placed into the flask and then heated on a hot stir plate (speed was set at 7-8, and the temperature was set at 6). 3 ml of ethidium bromide was added to the mixture. The solution was heated until all the agarose had dissolved (i.e. the clear liquid phase), which usually took 25 – 30 minutes.
4. The gel solution was cooled for 10 minutes before pouring into the gel tray in a cold room, and the combs were inserted with particular care to ensure they lined up straight.
5. The gel was left in room temperature until it solidified, which usually took 25 – 30 minutes.

4.3.6.1.2.3.2) TBE BUFFER

Concentrated TBE (x 10) stock was made by dissolving 108 g of Tris base, 55 g of Boric Acid and 7.44 g of EDTA in 700 ml of de-ionised distilled water and then made up to 1 litre with de-ionised distilled water. This was then stored at room temperature until use. To re-constitute the 1x TBE, 10 ml of the TBE

hyperconcentrate (x 10 stock) was mixed with 90 ml of de-ionised distilled water immediately prior to use.

4.3.6.1.2.3.3) Gel Loading Buffer (Blue) For Agarose Gels

50 ml of bromophenol blue (5 mg/ml) was mixed with 400 mg of sucrose and 950 ml of de-ionised distilled water. The mixture was vortexed at high speed and stored at 4°C.

4.3.6.1.2.3.4) 1 kb Ladder Marker

50 ml of the 1 Kb ladder, which is available through Invitrogen (Cat. Number 15615-016), was dissolved in 100 ml bromophenol blue and 350 ml of de-ionised distilled water. The solution was thoroughly mixed together before being separated into aliquot and stored at – 20°C.

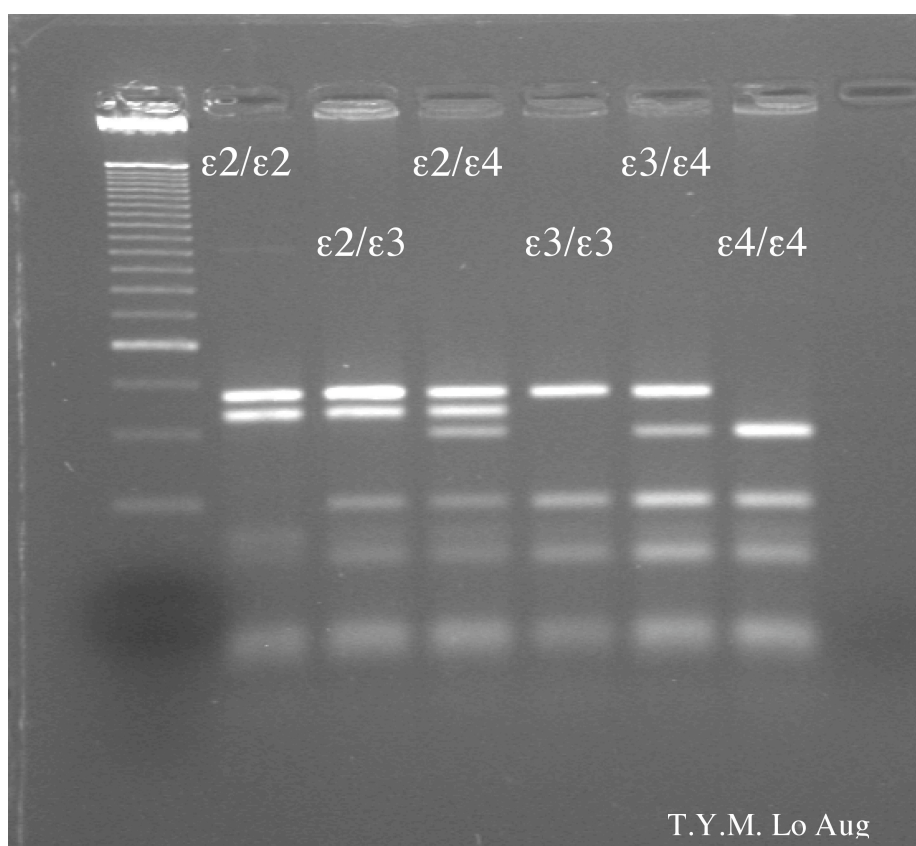
4.3.6.1.3) Restriction Enzyme Digest On PCR Products

1 µl of the restriction enzyme Hha1 (available through New England Biolabs Inc, Cat. Number R0139L) was added to each of the positive PCR product and the mixture was incubated overnight at 37°C using a vibrating water bath.

4.3.6.1.4) APO E Genotype Identification Using Gel Electrophoresis

Gel electrophoresis of the digested PCR product was performed to identify the different APO E genotypes. Fine separation was required for this gel electrophoresis and 4% metaphor agarose gel was used. 8 μ l of the gel loading buffer was mixed with the PCR digested product (40 μ l) before loading into the wells. 8 μ l of the 25 Kb ladder marker was used. The gel was allowed to run for one and a half hours using 120V. It was then read under UV light. Figure 4.1 shows a reference gel with the characteristic bands for the 6 APO E genotypes.

Figure 4.1:



4.3.6.1.4.1) 4% Metaphor Agarose Gel

To prepare one 4% metaphor agarose gel tray with 30 wells, the following processes were carried out:

1. 125 ml of 1x TBE was added to a flask.
2. 5g of metaphor agarose powder was added slowly in small portions while shaking the flask gently to prevent formation of clumps.
3. The flask was shaken until all metaphor agarose powder had dissolved into a thick solution.
4. A stir bar was added to the flask. The flask was then placed on a heated stir plate (the speed was set at 7-8, and temperature of the plate was set at 6). 4 ml of ethidium bromide was added to the mixture after 30 minutes. The solution was heated until bubbling and the clear liquid phase was achieved i.e. all the metaphor agarose powder had completely dissolved and this usually took 35 to 40 minutes. Care was taken to ensure the solution was not heated for too long because of the flux.
5. The gel solution was cooled off for 10 minutes before pouring (in a cold room) onto the gel tray with care while ensuring the combs were lined up straight.
6. The gel was left in room temperature until it solidified, which usually took 25 – 30 minutes. It was then placed in the fridge (+ 4°C) for 30 minutes before use to achieve a firmer consistency.

4.3.6.1.4.2) Gel Loading Buffer (Orange) For Metaphor Agarose Gel

50 ml of the Orange G (5 mg/ml) was mixed with 400 mg of sucrose and 950 ml of de-ionised distilled water. The solution was vortexed at high speed before being separated into aliquot and stored at 4⁰C until use.

4.3.6.1.4.3) 25 Kb Ladder Marker

40 ml of the 25 Kb DNA ladder marker (available through Invitrogen Cat. Number 10597-011) was mixed with 220 ml of bromophenol blue (5 mg/ml) and 500 ml of de-ionised distilled water. It was separated into small aliquot and stored at – 20⁰C until use.

4.3.7) Data Analyses

4.3.7.1) APO E Allelic Distributions

The frequency of the six APO E genotypes was determined for the brain injured children and their controls. The distribution ratios of the three APO E alleles were then calculated for these two groups of children. Comparisons of the APO E allelic distribution ratios were made among the brain injured children, their controls, and a previously reported cohort of healthy Scottish adults (n = 400) [257]. Chi square tests were employed to assess any statistical difference.

4.3.7.2) Allelic Dichotomies & Outcome Groups

The brain-injured children were divided into the following allelic dichotomies for analyses:

- (i) Patients possessing the APO E ϵ 2 allele vs. those without the APO E ϵ 2 allele
- (ii) Patients carrying the APO E ϵ 4 allele vs. those without the APO E ϵ 4 allele
- (iii) Patients who were APO E ϵ 3 homozygous vs. those who were not ϵ 3 homozygous.

The PICU discharge conscious states of the patients were dichotomised into those who had ‘regained consciousness’ (GCS > 8) and those with ‘delayed return of consciousness’ (GCS 8 or less). For the 6-month outcome, patients were grouped into ‘good recovery’ when GOS 4 and 5 was achieved, and ‘poor outcome’ when GOS was between 1 and 3.

4.3.7.3) APO E Alleles and Outcome

To assess the effects of the different APO E alleles on PICU discharge conscious state and outcome after childhood brain trauma, two-by-two tables were constructed between the different allelic dichotomies and outcome groups described above. Fisher’s Exact Tests were employed to detect statistical significance ($p < 0.05$).

4.3.7.4) APO E Alleles, Demographic Details, Injury Severity, and Secondary Insults

Statistical differences of demographic details, injury severity and secondary physiological derangement between the different allelic groups were assessed using Mann Whitney U tests (for continuous variables) or Fisher's Exact Tests (for nominal variables).

4.3.7.4.1) APO E Alleles and Age-Related CPP Insult

The relationship between of the total burden of CPP insult as measured by the cumulative pressure-time index and the different APO E alleles was determined using the Mann Whitney U test for the different allelic dichotomies.

4.3.7.5) APO E Alleles, Demographic Details, Age-related Physiological Derangements, and Outcome

Mann Whitney U tests were employed to detect difference in the frequency and duration of age-related physiological derangements, the total burden of CPP insult for the following APO E genotype and outcome dichotomies:

- Children in possession of the APO E e2 allele with 'Good recovery' vs. those in possession of the APO E e2 allele with 'Poor recovery'
- Children in possession of the APO E e4 allele with 'Good recovery' vs. those in possession of the APO E e4 allele with 'Poor recovery'

- Children in possession of the APO E e3 allele alone with ‘Good recovery’ vs. those in possession of the APO E e3 allele alone with ‘Poor recovery’

Logistic regression analyses were performed to determine the associations between the different APO E alleles on PICU discharge conscious state and outcome controlling for age, sex, post-resuscitation/pre-intubation GCS, initial brain CT findings, the duration of ICU stay and ICP monitoring, and age-related CPP insult as measured by PTIc. Post-resuscitation/pre-intubation GCS, initial brain CT findings and duration of ICU stay were included in the analyses because they are generally accepted as indicative of primary brain injury while CPP insult was the most significant and influential secondary insult on outcome. These key prognostic factors were agreed in advance. Age, duration of ICU stay, duration of ICP monitoring and PTIc were included in the regression model as continuous variables while the post-resuscitation GCS was categorised into severe injury where the GCS was 8 or less, and non-severe injury when the modified GCS was more than 8. Initial brain CT findings were classified into either diffuse or focal injuries for the purpose of this analysis.

4.4) RESULTS

4.4.1) Demographics

Of the sixty-five brain injured children (46 boys and 19 girls with a median age of 9.3 years), 2 suffered a mild injury (GCS 13 – 15), 25 suffered a moderate injury (GCS 9 – 12), while 38 had a severe injury (GCS \leq 8).

The subgroup of children with CPP insult measurement had more severe brain injury and longer duration of stay in ICU than those of the whole cohort but other demographic details such as age, sex distribution, and initial brain CT findings did not otherwise differ significantly (Table 4.1).

Table 4.1

	Whole Group			With CPP Insult Measurement		
	e2 present (n = 16)	e3 homozygous (n = 38)	e4 present (n = 14)	e2 present (n = 12)	e3 homozygous (n = 20)	e4 present (n = 9)
Median age in yrs (Mean age in yrs)	9.13 (9.03)	10.00 (8.90)	7.13 (7.56)	9.54 (10.19)	11.25 (10.04)	8.00 (8.20)
Sex: Boys Girls	12 4	29 9	7 7	9 3	17 3	6 3
Injury Severity: Minor Moderate Severe	1 4 11	1 15 22	0 6 8	0 3 9	0 5 15	0 3 6
Median Duration of PICU Stay in days (Mean in days)	5.50 (6.94)	5.00 (7.82)	5.00 (7.00)	6.50 (8.33)	7.50 (8.60)	5.00 (8.44)

4.4.2) APO E Allelic Distributions

APO E genotyping was successful in all participants. The APO E allelic distributions for brain injured children and their controls are summarised in Table 4.2. The distribution ratios of the three APO E alleles were similar between the brain injured children and their controls, but when compared with healthy adults from a previously reported Scottish population [257], the e2 allele was significantly over-represented ($p = 0.04$) among our participants (Table 4.2).

Table 4.2:

APO E Alleles						
	e2	e3	e4	Total		
TBI Children (whole cohort n = 65)	18 (0.14)	96 (0.74)	16 (0.12)	130		
Control Children (n = 160)	36 (0.11)	238 (0.74)	46 (0.15)	320		
χ^2 for trend: e2 p = 0.44; e3 p = 0.91; e4 p = 0.86						
APO E Alleles						
	e2	e3	e4	Total		
TBI Children (Whole cohort n = 65)	18 (0.14)	96 (0.74)	16 (0.12)	130		
TBI Children (CPP insult Measured n = 38)	13 (0.15)	53 (0.73)	10 (0.12)	76		
χ^2 for trend: e2 p = 0.53; e3 p = 0.53; e4 p = 0.86						
APO E Alleles						
	e2	e3	e4	Total		
TBI Children (Whole cohort n = 65)	18 (0.14)	96 (0.74)	16 (0.12)	130		
Healthy Adults (n = 400)*	66 (0.08)	616 (0.77)	118 (0.15)	800		
χ^2 for trend: e2 p = 0.04 ; e3 p = 0.43; e4 p = 0.46						
APO E Genotypes						
	e2/e2	e2/e3	e2/e4	e3/e3	e3/e4	e4/e4
TBI Children (whole cohort n = 65)	2	11	3	38	9	2
TBI Children (CPP insult Measured n = 38)	1	8	3	20	5	1
Control Children (n = 160)	3	26	4	90	32	4
Healthy Adults (n = 400)*	2	51	11	233	99	4

*[239]

4.4.3) APO E Alleles and Outcome

Of the whole brain trauma cohort, 46 children ‘regained consciousness’ while 19 remained in a coma at the time of PICU discharge. At 6 months post injury, 8

children had ‘poor outcome’ of whom only 1 had ‘regained consciousness’ at PICU discharge ($p < 0.001$, Fisher’s Exact Test).

Table 4.3 summarises the relationships between the different allelic dichotomies and outcome at PICU discharge and 6 months post injury. Only 3 of the 38 e3 homozygous (8%) had ‘poor outcome’ at 6 months post injury while 3 of the 16 e2 carriers (19%) and 3 of the 14 e4 possessors (21%) had ‘poor outcome’. There was a trend for the e2 allele possessors to remain in a coma at PICU discharge ($p = 0.05$, Fisher’s Exact Test) (Table 4.3).

Table 4.3:

	Regained Consciousness (n = 46)	Delayed Return of Consciousness (n = 19)	Significance (Fisher’s Exact Test)
Whole Cohort:			
e2 allele present	8	8	$p = 0.05$
No e2 allele present	38	11	
e3 homozygous	30	8	$p = 0.10$
Non-e3 homozygous	16	11	
e4 allele present	8	6	$p = 0.32$
No e4 allele present	38	13	
	Good Recovery (n = 57)	Poor Outcome (n = 8)	Significance (Fisher’s Exact Test)
Whole Cohort:			
e2 allele present	13	3	$p = 0.40$
No e2 allele present	44	5	
e3 homozygous	35	3	$p = 0.26$
Non-e3 homozygous	22	5	
e4 allele present	11	3	$p = 0.35$
No e4 allele present	46	5	

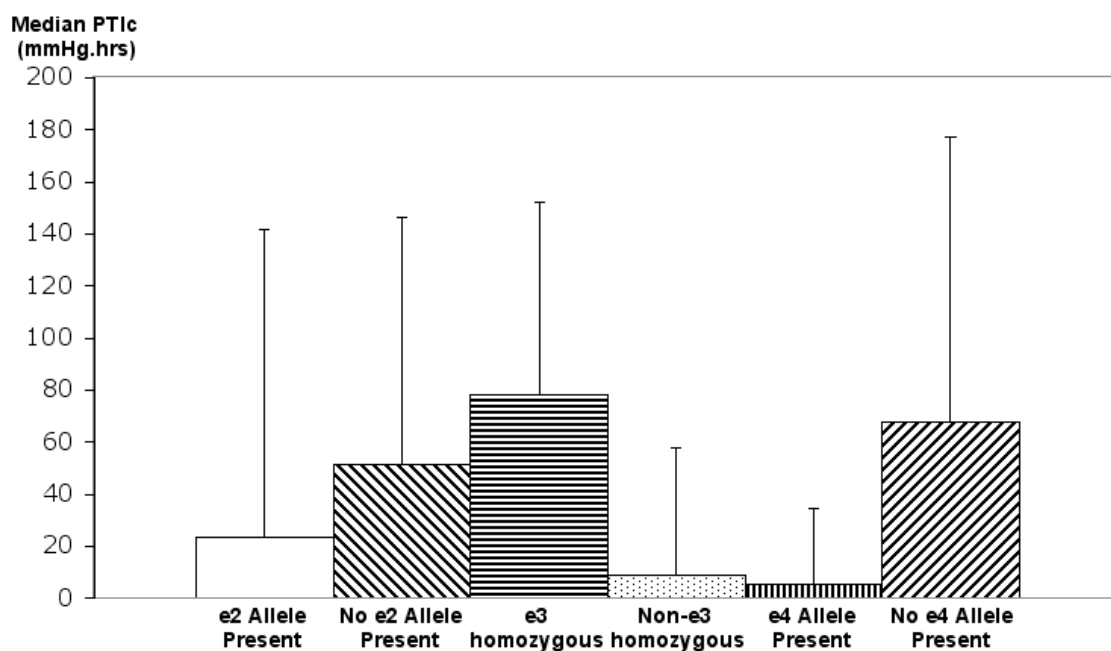
4.4.4) APO E Alleles and Secondary Derangement

The frequency and duration of secondary age-related physiological derangements did not differ between children of the different allelic groups.

4.4.4.1) APO E Alleles and The Total Burden of CPP Insult

Children with various APO E alleles experienced differential amounts of CPP insult with the e3 homozygous experiencing the most while the e4 carriers had the least CPP insult. Children carrying the e4 alleles had significantly less (13.3 times) CPP insult than those without the e4 allele ($p = 0.04$, Mann Whitney U Test) (Figure 4.2). E3 homozygous suffered 9.2 times more CPP insult than the non-e3 homozygous ($p = 0.03$, Mann Whitney U Test) (Figure 4.2). In general, children in possession of the e2 allele experienced 2.2 times less CPP insult than the non-e2 carriers but this did not reach statistical significance ($p = 0.58$, Mann Whitney U Test) (Figure 4.2).

Figure 4.2:



4.4.5) APO E Alleles, CPP Insult and Outcome

4.4.5.1) At PICU Discharge

Children with delayed recovery of consciousness at PICU discharge tended to have experienced more CPP insult than those who regained consciousness early with the exception of those who were e3 homozygous where little variation of CPP insult was found (Table 4.4).

Table 4.4:

	Median PTIc in mmHg.hrs (ranges)		Significance (Mann Whitney U)
	Regained Consciousness	Delayed Return of Consciousness	
Whole Group	33.03 (0.00–273.22) (n = 22)	60.83 (0.00–1169.27) (n = 16)	$p = 0.30$
e2 allele present	2.22 (0–13.77) (n = 4)	82.47 (0–1169.27) (n = 8)	$p = 0.06$
e3 homozygous	75.26 (2.27–273.22) (n = 14)	79.95 (0.00–830.40) (n = 6)	$p = 0.93$
e4 allele present	1.33 (0.00–11.93) (n = 4)	22.83 (0.00–115.95) (n = 5)	$p = 0.14$
	Median PTIc in mmHg.hrs (ranges)		Significance (Mann Whitney U)
	Good Recovery	Poor Outcome	
Whole Group	32.33 (0.00–388.58) (n = 31)	150.20 (5.10–1169.27) (n = 7)	$p = 0.04$
e2 allele present	4.43 (0.00–121.28) (n = 9)	150.20 (48.98–1169.27) (n = 3)	$p = 0.03$
e3 homozygous	70.13 (0.00–388.58) (n = 18)	495.86 (161.32–830.40) (n = 2)	$p = 0.04$
e4 allele present	1.33 (0.00–115.95) (n = 6)	22.83 (5.10–48.98) (n = 3)	$p = 0.20$

When considering children who regained consciousness (GCS > 8) at PICU discharge, e3 homozygous had suffered significantly more CPP insult than the non e3 homozygous ($p < 0.01$, Mann Whitney U Test) while children with the e4 allele experienced significantly less CPP insult than those without the e4 allele ($p = 0.03$, Mann Whitney U Test) (Table 4.5). There was a trend to suggest that the e2 carriers who had ‘regained consciousness’ had suffered less CPP insult than those without the e2 allele ($p = 0.05$, Mann Whitney U Test). (Table 4.5)

Table 4.5:

	Median PTIc in mmHg.hrs (ranges)		<i>p</i> value
	e2 present	No e2 present	
Regained consciousness (PICU discharge)	2.22 (0.00–13.97) (n = 4)	51.17 (0.00–273.22) (n = 18)	<i>p</i> = 0.05
Good recovery (6 months post injury)	4.43 (0.00–121.28) (n = 9)	51.17 (0.00–388.58) (n = 22)	<i>p</i> = 0.23
Poor outcome (6 months post injury)	150.20 (48.98–1169.27) (n = 3)	92.08 (5.10–830.40) (n = 4)	<i>p</i> = 0.48
	Median PTIc in mmHg.hrs (ranges)		<i>p</i> value
	e3 homozygous	Non-e3 homozygous	
Regained consciousness (PICU discharge)	75.26 (2.23–273.22) (n = 14)	1.33 (0.00–13.77) (n = 10)	<i>p</i> < 0.01
Good recovery (6 months post injury)	70.13 (0.00–388.58) (n = 18)	2.67 (0.00–121.28) (n = 13)	<i>p</i> = 0.02
Poor outcome (6 months post injury)	495.86 (161.32–830.40) (n = 2)	48.98 (5.10–1169.27) (n = 5)	<i>p</i> = 0.25
	Median PTIc in mmHg.hrs (ranges)		<i>p</i> value
	e4 present	No e4 allele	
Regained consciousness (PICU discharge)	1.33 (0.00–11.93) (n = 4)	51.17 (0.00–273.22) (n = 18)	<i>p</i> = 0.03
Good recovery (6 months post injury)	1.33 (0.00–115.95) (n = 6)	49.95 (0.00 – 488.58) (n = 25)	<i>p</i> = 0.09
Poor outcome (6 months post injury)	22.83 (5.10–48.98) (n = 3)	495.86 (150.20–1169.27) (n = 4)	<i>p</i> = 0.03

4.4.5.2) 6 Months Post Injury

At 6 months post injury, regardless of the APO E genotypes, brain injured children with a good recovery had suffered less CPP insult than those who had poor outcome (Table 4.4). When considering children with a good recovery, e3 homozygotic patients recovered well despite having suffered significantly (nearly 26 times) more CPP insult during their ICU management than those who were not e3 homozygous ($p = 0.02$, Mann Whitney U Test) (Table 4.5). Children possessing the e4 allele with a poor outcome experienced significantly less CPP insult than those without the e4

allele ($p = 0.03$, Mann Whitney U Test) (Table 4.5). Furthermore, the median PTIc for the e4 carriers with poor outcome was 22.8 mmHg.hr which should have conferred good recovery given that the median PTIc for the whole cohort with good recovery was 32.3 mmHg.hr (Table 4.4).

4.4.6) Determinants of Outcome after Childhood Brain Trauma

4.4.6.1) Delayed Return of Consciousness at PICU Discharge

Different APO E alleles had different associations with the PICU discharge conscious status (Table 4.6). The presence of the e2 allele was associated with ‘delayed return of consciousness’ at PICU discharge ($p = 0.0146$, 95% CI 0.001 – 0.472). A negative association was found with the e3 homozygous ($p = 0.0253$, 95% CI 1.629 – 1616.765) but the possession of the e4 allele had no association with PICU discharge GCS of 8 or less.

Age was associated with delayed returned of consciousness at PICU discharge in children who were e3 homozygous ($p = 0.0337$, 95% CI 1.015 – 1.441) and those possessing the e4 allele ($p = 0.0336$, 95% CI 1.019 – 1.458), but it did not appear to have any association with the PICU discharge conscious state in the e2 allele carriers. No correlation was demonstrated between CPP insult and the coma status at PICU discharge.

4.4.6.2) Poor Outcome at 6 Months Post Injury

Poor outcome at 6 months after childhood brain trauma was significantly related to the amount of CPP insults experienced during neuro-intensive care regardless of the child's APO E genetic composition (Table 4.7). Possession of the e4 allele had a positive association ($p = 0.0325$, 95% CI 1.471 – 1782.500) with poor outcome while being an e3 homozygous had a negative correlation ($p = 0.0010$, 95% CI 1.376 – 1.609) with poor outcome (Table 4.7). No significant association was demonstrated between poor outcome at 6 months post injury and the possession of the e2 allele.

Table 4.6

Factors Associated with Delayed Return of Consciousness at PICU Discharge

	Coefficiency	Significance (p value)	95% Confidence Intervals
e2 allele present	3.807	$p = 0.0146$	0.001 – 0.472
Sex (Boys)	- 5.905	$p = 0.0381$	1.381 – 97469.601
Age	0.301	$p = 0.1409$	0.495 – 1.105
Severe injury	1.056	$p = 0.4439$	0.023 – 5.194
Initial brain CT (focal injury)	2.457	$p = 0.1059$	0.004 – 1.685
Duration of PICU stay	1.198	$p = 0.0304$	1.009 – 1.667
CPP insult (PTIc)	0.003	$p = 0.4142$	0.990 – 1.004
e3 homozygous	- 3.938	$p = 0.0253$	1.629 – 1616.765
Sex (Boys)	- 5.478	$p = 0.0287$	1.766 – 32464.56
Age	1.402	$p = 0.0337$	1.015 – 1.441
Severe injury	1.561	$p = 0.2594$	0.014 – 3.164
Initial brain CT (focal injury)	2.356	$p = 0.0997$	0.006 – 1.567
Duration of PICU stay	0.249	$p = 0.0384$	0.616 – 0.987
CPP insult (PTIc)	0.005	$p = 0.1603$	0.987 – 1.002
e4 allele present	1.946	$p = 0.1298$	0.012 – 1.771
Sex (Boys)	- 4.776	$p = 0.0045$	1.932 – 7289.863
Age	0.382	$p = 0.0336$	1.019 – 1.458
Severe injury	1.112	$p = 0.4165$	0.022 – 4.812
Initial brain CT (focal injury)	0.837	$p = 0.4650$	0.046 – 4.092
Duration of PICU stay	0.147	$p = 0.0631$	0.730 – 1.019
CPP insult (PTIc)	0.003	$p = 0.4334$	0.991 – 1.004

Table 4.7:**Factors Associated with Poor Outcome at 6 month Post Injury**

	Coefficiency	Significance (<i>p</i> value)	95% Confidence Intervals
e2 allele present	2.156	<i>p</i> = 0.2024	0.213 – 350.605
Sex (Boys)	-1.379	<i>p</i> = 0.3093	0.018 – 3.595
Age	0.139	<i>p</i> = 0.4937	0.772 – 1.711
Severe injury	0.952	<i>p</i> = 0.6105	0.009 – 15.722
Initial brain CT (focal injury)	2.945	<i>p</i> = 0.0952	0.343 – 105.104
Duration of PICU stay	0.184	<i>p</i> = 0.0893	0.949 – 1.523
CPP insult (PTIc)	1.009	<i>p</i> = 0.0184	1.019 – 1.999
e3 homozygous	-9.06	<i>p</i> = 0.0010	1.376 – 1.609
Sex (Boys)	-0.745	<i>p</i> = 0.6925	0.012 – 19.055
Age	0.739	<i>p</i> = 0.1216	0.821 – 5.341
Severe injury	0.565	<i>p</i> = 0.8358	0.003 – 118.928
Initial brain CT (focal injury)	6.930	<i>p</i> = 0.0776	0.464 – 225.499
Duration of PICU stay	0.407	<i>p</i> = 0.0797	0.953 – 2.369
CPP insult (PTIc)	1.029	<i>p</i> = 0.0003	1.059 – 1.999
e4 allele present	3.366	<i>p</i> = 0.0325	1.471 – 1782.500
Sex (Boys)	-0.918	<i>p</i> = 0.5837	0.015 – 10.649
Age	0.266	<i>p</i> = 0.2646	0.817 – 2.083
Severe injury	0.106	<i>p</i> = 0.9615	0.015 – 84.717
Initial brain CT (focal injury)	1.984	<i>p</i> = 0.9615	0.360 – 146.939
Duration of PICU stay	0.138	<i>p</i> = 0.214	0.911 – 1.447
CPP insult (PTIc)	1.010	<i>p</i> = 0.0046	1.102 – 1.999

4.5) DISCUSSIONS

Potential genetic influence on neurological recovery after brain trauma was first described in 1995. Since then, many clinical studies have emerged in the literature linking the APO E e4 allele possession with poorer outcome after brain trauma in adults.

Teasdale and co-workers prospectively examined the associations between APO E genotypes and the 6-month global outcome score of 93 head injured patients, who

were predominantly adults, and found that twice as many in possession of the e4 allele made a poor recovery as those without this particular allele [141].

Friedman and colleagues investigated the predictive value of the APO E genotypes on the duration of unconsciousness and functional outcome in 69 adults after head injury, but took no account of the genetic influence on non-survivors [143]. Carriers of at least one APO E e4 allele in their cohort were more likely to remain unconscious for more than 7 days [143]. In addition, only 3% of the e4 carriers had a good functional recovery at 6 – 8 months post injury whereas 10 times as many non-e4 carriers recovered with a good function outcome [143]. Duration of unconsciousness is likely to be largely reflective of the primary brain injury and not a measure of secondary brain insults, but yet these investigators found that the possession of the APO E e4 allele were associated with longer duration of unconsciousness and poorer outcome. This is suggestive that APO E genetic polymorphisms may differentially affect patients' response to primary brain injury, which has contributed to the length of coma.

In a further study which excluded fatal brain trauma, Lichtman's group reported on the functional recovery in 31 surviving adults of brain trauma and found poorer outcome among the APO E e4 carriers [142]. APO E e4 allele might also have a negative influence on late outcome of brain trauma as described in a recent study evaluating the specific outcome measure (neuropsychological outcome) of 396 survivors of head injury [258]. They found 1.3 times more non-e4 carriers had a good long-term neuropsychological outcome than the e4 possessors [258].

4.5.1) Over-Representation of the e2 Allele in Active Children With or Without Brain Injury

The patients in the previously reported APO E brain trauma studies consisted of either exclusively or predominantly adults with very few paediatric cases. Although the APOE allelic distribution ratios from all these cohorts [141, 259, 260] were reported to be similar to previously published population data, none have actually included appropriate population controls for their injured cohorts. Our study evaluated the associations between the child's APO E genotype, the measured secondary physiological derangements after brain injury, and outcome classification at ICU discharge and 6 months post injury [261]. To the best of my knowledge, this is the first clinical study within the APO E-brain trauma literature to investigate APO E genotypes' influence on secondary insult and outcome after brain trauma in an exclusive child cohort which includes an appropriate same population control group.

Our control subjects were comprised of healthy active children who had sustained minor injuries from their normal life styles and were age and sex matched to the brain-injured group. They were chosen to ensure as similar a risk for sustaining brain injury as possible to allow accurate comparison of the APO E allelic distribution frequency which we found to be similar between the groups [261]. Our decision to include a separate paediatric population control group was further justified by the finding of a significant e2 allelic over-representation among our participants when compared to that of a previously reported group of healthy adults

who were born in North-East Scotland [257]. This adult cohort was chosen for comparison because it represented the largest reported healthy Scottish adult cohort to have their APO E genotyped and its allelic distribution ratio (0.08 for e2, 0.77 for e3, and 0.15 for e4) was similar to those described in other adult brain trauma cohorts [141, 259, 260], and general populations worldwide [247, 262] including different regions of Scotland, Germany, Taiwan, and the United States of America. Geographic variation of the APO E allelic distribution is, therefore, unlikely to explain our observed over-representation of the e2 allele.

The frequency of the e2 allele among a previously reported healthy Scottish newborn population [263] recruited from the same geographic area as our cohort, was similar to the e2 allele frequency of the above adult reports rather than to our study. Our participants, children with minor injuries and critically ill children post brain trauma, may, therefore, represent a different population to brain injured adults and the general (adult and neonatal) populations. Paediatric injuries tend to occur more frequently because of the level of activity among normal young children, while adult injuries (include the elderly population) may occur regardless of their activity levels. Further investigation is required to determine whether APO E e2 allele relates to a heightened physical activity level.

4.5.2) Different Amount of CPP insult between the Carriers of the Different APO E Alleles

Almost all previous clinical studies of APO E polymorphisms and brain trauma have focused on its influence on neurological recovery and using it as a potential prognostic indicator without making any serious attempts to determine how APO E genotypes may affect outcome. Although genetic constitution cannot be altered, understanding how genetic polymorphisms differentially modulate recovery after brain trauma may allow suggestions for novel therapies to be directed specifically to patients with or without a particular allele. This would be particularly useful in brain trauma management especially when no novel treatment has been introduced for the past 20 years despite extensive attempts to translate basic science research data into effective and novel treatment suitable for clinical use.

CPP insult is a proven determinant of outcome after brain trauma [63, 66, 204] and its prevention remains the main emphasis of all modern neuro-intensive care management strategies worldwide [74, 75]. Although CPP insult and APO E polymorphisms have independently been described to affect brain trauma outcome, the relationships between APO E genotypes and CPP insult following brain trauma has not been evaluated previously.

Our study confirms the importance of CPP insult on outcome after childhood brain trauma regardless of the patient's APO E status [261]. Additionally, this study was the first to report children carrying the e4 allele to have experienced the least amount

of CPP insult, while the e3 homozygotic patients suffered the most after brain trauma despite having sustained primary brain injury of similar severity and being treated with standardised intensive care management [261]. This suggests that APOE E genetic polymorphisms may potentially affect the body's response to primary brain injury or its management differently.

Our patients possessing the e4 allele with poor outcome had experienced a lesser burden of CPP insult than non-e4 carriers and the amount of CPP insult was at a level that should have conferred good recovery [261]. Carriers of the e4 allele may be less tolerant of CPP insult and develop more cerebral ischaemic damage than children without this allele so that even with the small amount of CPP insult they suffered, which should have conferred good recovery, the e4 carriers had poor outcome. This interpretation would support the observed trend of an increased incidence of severe ischaemic brain damage among the e4 carriers from a recent report of a post-mortem study evaluating brain specimens from 239 fatal cases of TBI aged between 2 months and 84 years [264] although the burden of secondary CPP insult suffered by these patients were unknown.

Cerebral ischaemia may be caused by an imbalance of the cerebral metabolic demand and substrate delivery. Lesser amount of CPP insult found in our e4 carriers would imply the presence of age-appropriate cerebral perfusion. It is known that cerebral metabolic rate in severely brain injured children was normal in 81% and this subsequently fell between the first and third day following brain trauma [89]. One may, therefore, postulate that the e4 allele increases its carriers' cerebral metabolic

demand after brain trauma and thereby increasing their susceptibility to cerebral ischaemia. To assess this postulate, continuous measurements of cerebral metabolism and cerebral substrate supply would be required.

Recent animal studies have suggested that brain trauma causes an increased neuro-inflammation in transgenic animals carrying the human e4 allele when compared with those with the e3 allele [265, 266]. It is unknown whether humans with different APO E alleles experience a different degree of neuro-inflammation and further studies are required to ascertain whether this may explain our observation on outcome which appeared out of proportion to the allelic-related variations in CPP insult post brain trauma. Another possible explanation for this observation is that the APO E e4 allele adversely affects outcome through mechanisms independent of the amount of CPP insult.

Almost all previous reports in the literature have suggested the adverse effect of the APO E gene on outcome was related to the possession of the e4 allele. However, despite transgenic animal data suggesting a potentially more superior neurological repair offered by the e3 allele [251], no study has investigated whether APO E allelic influence on neurological recovery may be related to the absence of the e4 allele. The e3 homozygotic children in our cohort experienced more CPP insult than those in the other allelic groups which by conventional evidence on the positive correlation between CPP insult and poor outcome [63, 66, 204], more of the e3 homozygous would have been expected to have an unfavourable outcome. However, we found fewer e3 homozygotic patients among the brain injured children with a poor

outcome. Additionally, children possessing only the e3 allele with ‘good recovery’ in our cohort had achieved that despite having suffered nearly 26 times more CPP insult than non-e3 homozygous [261]. This would suggest the e3 homozygous potentially enjoys a protective effect from ischaemic insult. This benefit may be due to the lack of the e4 or e2 alleles and warrants further investigations.

4.5.3) APO E Polymorphisms Influence Recovery at Different Time Point Post Injury

Most studies in the APO E genetic polymorphisms – brain trauma literature assessed outcome at a single time point post-injury. In a recent adult study where the e4 allelic influence on the speed of recovery after brain trauma was evaluated, Alexander and colleagues reported individuals possessing the e4 allele recovered at a significantly slower rate than those without the allele [260]. They additionally found that the e4 carriers who survived the initial injury and early recovery phase had poorer long-term outcome at 24 months post injury than those without the e4 allele [260]. Effect of the other APO E alleles on the recovery rate was, however, not assessed in their report.

We found that different APO E alleles had different associations with the PICU discharge conscious state and the 6-month global outcome. Although the presence of the e4 allele had no association with the PICU discharge coma status, it was associated with poor outcome at 6 months post injury. This suggests that the e4

allele may have more influence on longer term recovery, which is consistent with the findings reported by Alexander and co-workers [260] as described above.

Possession of the e2 allele was significantly associated with the delayed return of consciousness at PICU discharge in our cohort, but it was not associated with poor outcome at 6 months post injury. One possible interpretation of this finding is that the e2 allele may have a negative influence on the early recovery from brain trauma. However, one previous family study of Alzheimer's disease suggested the possession of the e2 allele lowered the risk of developing Alzheimer's Disease [249]. If the APO E e2 allele potentially offers protection against neuro-degenerative disease, it is, therefore, possible to suggest that it may have a similar protective effect on neurological recovery after brain trauma but its protective effect does not take place until later in the recovery process. Given the rarity of this allele among the general population and the absence of a transgenic APOE e2 animal model, a large multi-centre study with appropriate controls from each centre would be necessary to assess the true effect of APOE e2 allele on neurological recovery after brain trauma.

Being an e3 homozygous had a negative association with both delayed returned of consciousness at PICU discharge and poor outcome at 6 months post injury in our study. This supports the postulation that the e3 allele offers a more superior recovery from brain trauma as observed in transgenic animal studies [251]. Alternatively, the beneficial effect on recovery may be the result of the lack of either e4 or e2 allele. This highlights the need to assess the effects of all APO E alleles on the recovery

after brain trauma at various time points without restricting the investigation to only the e4 allele.

4.5.4) Limitation of the Study

Although we have demonstrated carriers of the various APO E alleles experienced a differential burden of CPP insult, the small cohort size has limited any possibility of ascertaining whether the presence or absence of any particular allele has caused this observation.

The proportion of patients with poor outcome was unexpectedly low in our cohort, and was a third less than the projected figure from previous reports [141]. Our original power calculation was based upon figures available from these previously published adult studies with a similar projected proportion of unfavourable outcome. Successful recruitment of 65 patients should have produced a 5% significance value when assessing the influence of the different APOE alleles on outcome. Given a more than 50% reduction in the number of patients with unfavourable outcome, to achieve a 5% significance would require twice the number of patients recruited which was not possible within the planned time frame since all suitable patients had been included. Fewer unfavourable outcomes following childhood brain trauma, although a welcome finding, makes our study insufficiently powered to assess whether there is any allelic influence on neurological recovery after childhood brain trauma.

Despite these limitations, we demonstrated that unfavourable outcome at 6 months was 2.6 times more common among the e4 carriers than the e3 homozygotic patients, although 58% of the cohort were e3 homozygous and only 22% were e4 carriers, who suffered the least amount of CPP insult. We additionally demonstrated that different APO E alleles potentially exert their influence on recovery at different phases after brain trauma. The e3 homozygous appeared to enjoy a sustained positive influence on recovery starting at the PICU discharge to 6 months post injury, while the potential negative influence of the e4 allele only appeared to occur to late recovery. The paucity of our cohort size was again reflected in the wide confidence intervals for the e4 allelic association with poor outcome. Our findings highlighted the need to further investigate the influence of APO E alleles on childhood brain trauma. Additionally, our observed over-representation of the e2 allele in active children reaffirmed the importance to include an appropriate control population in any future studies.

4.6) CONCLUSIONS

After childhood brain trauma, carriers of the APO E e4 allele may tolerate CPP insult less well than those with other allelic possession. APO E e3 homozygotic patients may enjoy a relative protective effect, mitigating CPP insult.

CHAPTER 5: BRAIN TRAUMA SERUM BIOMARKERS AND THEIR PROGNOSTIC VALUES FOR UNFAVOURABLE OUTCOME AFTER BRAIN TRAUMA

5.1) BACKGROUND

Current methods to classify brain injury severity and to predict outcome during the acute management of brain injured patients remain limited. The most frequently used clinical method to classify brain injury severity continues to be the Glasgow Coma Scale (GCS) [28]. Although its inter-observer reliability is high among adult patients, GCS may be difficult to assign accurately in brain injured children, who are often frightened and uncooperative, especially if this is attempted by clinicians inexperienced in assessing the immature nervous system and may result in either over or under estimation of injury severity. Inaccurate classification of brain injury severity has serious consequence to the provision of the appropriate treatment required and may therefore, potentially affects recovery.

A number of biochemical mediators are produced after brain trauma when there is irreversible damage to the glial and neuronal cells [163-168]. Other biochemical mediators are produced to modulate the complex cascade of brain trauma induced secondary brain insult and pathophysiological repair processes [122, 123, 125, 126, 169-176]. Some of these mediators are measurable in blood or CSF, and their acute blood or CSF concentrations may, therefore, potentially be used to classify injury severity, to monitor recovery, or to predict outcome. Many potential brain

biochemical markers have been studied so far and may be classified into three major groups: (i) brain specific proteins; (ii) inflammatory mediators; and (iii) vasoconstrictors.

5.1.1) Brain Specific Proteins

Brain specific proteins are synthesized by the astroglial cells or neurons, and have been proposed as potential markers of glial and neuronal cell damage or disruption of the blood-brain barrier integrity following documentation of their detections in the CSF and the systemic circulation after central nervous system disorders or insults [163, 164, 267-270]. Numerous brain specific proteins have been investigated in brain injury but those that have attracted most research attentions included S-100B protein, neuron specific enolase (NSE), and creatine kinase (CK).

The S-100 protein is a small dimeric calcium binding protein found in the cytosol with a molecular weight of 22kD [271]. Depending on its alpha or beta chain structure, several forms exist. The $\beta\beta$ -form or the S-100B protein is found in high concentration in glial cells and Schwann cells, while the S-100A protein ($\alpha\beta$ -form) is present in glial cells only [272]. The S-100A0 or the $\alpha\alpha$ -form of the protein is found exclusively in neurons [272]. The S-100 protein can be found in much smaller concentration in non-nervous tissues such as adipose tissue, melanocytes and T-lymphocytes [273-276]. It is metabolised in the kidneys and excreted in the urine with a biological half-life of around 2 hours [277].

Neuron specific enolase (NSE) (defined as γ -subunit of enolase) is a soluble cytoplasmic protein localised principally in neurons and neuroendocrine cells [278-281]. Although its precise role in the nervous system remains unclear, recent evidence suggests it may have important interaction with the neuroinflammatory cascade [282, 283].

CSF and serum concentrations of these brain specific proteins are usually very low in normal individuals but become elevated following primary neurological disorders such as TBI [163, 164, 168, 284], stroke [283, 285, 286], seizures [287, 288], and secondary neurological complications after circulatory arrest [269, 289] and cardiopulmonary bypass [290]. After brain trauma, the concentration of the brain specific proteins in the CSF and serum rise steadily reaching a maximal level at the second day post injury and decline to undetectable values within 7 days.

The precise mechanisms by which these brain specific proteins are released into the CSF and bloodstream remain unclear but direct release into the CSF from damaged astrocytes and neurons followed by diffusion into the systemic circulation through a disrupted blood-brain barrier seems a likely mechanism given the positive correlation between the serum concentrations of these proteins and the size of the brain lesions [291-293]. This theory was supported by the findings of Pleines' group who demonstrated that the CSF concentrations of S100B and NSE in brain trauma patients were ten times higher than those detected in the serum samples collected simultaneously [294]. Thus, the blood or CSF concentrations of these brain specific

proteins measured during the acute management of brain trauma patients may be used as a marker of the severity of astroglial damage.

Elting and co-workers demonstrated the serum S-100B levels after strokes peaked 3 to 4 days later than those after brain trauma suggesting alternate release mechanisms existed following different brain insults [268]. In vitro studies have demonstrated that S-100B protein can be released into the extracellular space by (i) activation of A1 adenosine or mGlu3 metabotropic glutamate receptors [295], (ii) stimulation of astroglial 5-HT1A receptors [296-298], (iii) adrenocorticotrophic hormone (ACTH) and corticotrophin-like intermediate-lobe peptide [299], and (iv) secretion from proliferating astrocytes [300]. The extracellular adenosine level rises soon after experimental TBI [301, 302] and stroke [303] and is thought to be due to rapid intracellular ATP depletion resulting in an immediate secretion of S-100B. This mechanism offers a likely explanation to the acute rise in serum and CSF S-100B levels in human TBI patients but does not fit with the clinical observation in stroke. It has been suggested that following stroke high level of adenosine can be found within the core of the infarct, which is an area not perfused with blood resulting in accumulation of S-100B protein in the region without being released into the bloodstream. The delayed release of S-100B may then be explained by reactive astrogliosis which is observed to be at its maximal intensity on immunohistochemical staining approximately 3 to 4 days after induction of experimental TBI [304-306] and stroke [307, 308]. Most clinical studies have shown the serum and CSF S-100B levels begin to drop 2 days after brain trauma [166, 309, 310] indicating a lesser degree of reactive astrogliosis than those observed in stroke.

However, reactive astrogliosis is likely to play a role in brain trauma as well because a secondary excessive rise in serum S-100B levels had been observed in three severely brain injured adults, all with fatal outcome despite normal CPP, SaO₂, PaCO₂ and controlled ICP [311].

The mechanisms by which brain injury is sustained may also affect the temporal release pattern of S-100B and NSE [312]. Berger's group examined CSF samples collected from infants with an inflicted head injury and showed a peak level for both S-100B and NSE at a median of 63 hours after admission while those collected from children with accidental head injury showed a peak level at a median of 11 hours following trauma [312]. Furthermore, a second peak in NSE concentration was only observed in patients with inflicted head injury [312]. This is consistent with recent findings from experimental TBI animal models and clinical studies showing an increase in markers of delayed neuronal death in abuse victims [313-315].

Acute serum or CSF brain specific protein concentrations may provide an objective indication of the initial brain trauma severity [168, 293, 294, 310] but the predictive values of these proteins in outcome is less clear. Rothoerl and colleague examined 41 head injured adults with varying degree of severity and found that the mean serum S-100B levels were significantly higher in those with unfavourable outcome [168]. Another recent study suggested that only the admission serum S-100B correlated with outcome [316]. Among 84 severe brain trauma patients, the peak serum S-100B levels obtained from patients with favourable outcome was significantly lower than those with unfavourable outcome, but the serum NSE levels did not differ

between the two groups [317]. Others investigators had also failed to demonstrate a correlation between the serum or CSF NSE concentrations and recovery [166, 310].

Very little information is available in the literature on brain specific proteins and children with traumatic brain injury. Berger's group reported the CSF concentrations of these proteins in head injured children to be much higher than their adult counterparts suggesting a potential increased in susceptibility of the developing brain to neuronal or glial cell death after traumatic injury [312]. However, further studies are required to clarify the role of brain specific proteins as a marker of injury severity and predictor of outcome in children after brain trauma.

5.1.2) Inflammatory Mediators

5.1.2.1) Cytokines in Acute Brain Injury

Cytokines are low molecular weight polypeptides with a primary action to mediate inflammation and growth process. Brain injury stimulates microglia and astrocytes to produce and release cytokines such as interleukins (predominantly IL-6, IL-8, and IL-10) which are thought to be responsible for many of the clinical signs of inflammation observed after acute brain injury namely pyrexia, neutrophilia, and brain swelling. They are also thought to play a vital role in the production and release of other mediators in the secondary brain injury cascade such as oxygen-derived free radicals, neuropeptides and arachidonic acid derivatives, and up-regulation of adhesion molecules activities.

5.1.2.1.1) Interleukin 6 (IL-6)

IL-6 is an important mediator capable of causing and inhibiting inflammation following brain injury [109, 318]. In addition, its ability to increase endothelial permeability [319] suggests its potential involvement in the evolution of the blood-brain barrier disruption. Its presence in the brain following experimental traumatic brain injury was first reported in the early 1990s [320-323] with peak concentration found around 8 hours following the brain insult [322]. In vitro study using cultured human astrocyte has also shown an increased IL-6 production following fluid percussion injury [324].

Several clinical studies have investigated the role of IL-6 as a monitor of recovery following brain injury. McClain's group found an increased serum IL-6 concentration in 30 brain injured patients with the highest level observed on the first day after the injury [121]. The decline in serum IL-6 level correlated with clinical improvement and the rate of reduction in patients with an admission GCS 8-10 was faster than those with an admission GCS <8 [121]. This release of IL-6 soon after brain trauma has also been confirmed to occur in children. Bell and co-workers studied paired CSF and serum IL-6 levels in severely brain injured children with GCS \leq 8 and found raised IL-6 concentration in both fluid with a peak level demonstrated within the first day of injury [122]. In another study of 45 children with TBI, Kalabalikis and colleagues have demonstrated the peak serum IL-6 level occurred 4 hours post injury and declined steadily over the next three days [123].

The IL-6 level of head injured children at 72 hours post injury remained higher than those of healthy controls [123]. Although the IL-6 concentration was significantly higher in the severely injured children (GCS < 8), they did not demonstrate any correlation with clinical outcome [123].

Experimental studies have suggested that IL-6 detected after brain trauma is likely to have originated intracranially. Elevated IL-6 concentration in ventricular fluid had been documented to occur after brain trauma in both adults [121, 124] and children [122]. McKeating and co-workers from Edinburgh investigated the arterial and jugular venous IL-6 levels in 32 adults with either TBI or spontaneous subarachnoid haemorrhage (SAH) [171]. They demonstrated a marked jugular venous-arterial difference in the elevated IL-6 concentration supporting the possibility of intracranial IL-6 production following brain injury [171].

5.1.2.1.2) Interleukin 8 (IL-8)

Interleukin 8 (IL-8) is a pro-inflammatory cytokine belonging to the cysteine-x-cysteine family with potent chemotactic and activating properties on neutrophils [109]. It is synthesised by various cell types including monocytes/macrophages, endothelial cells, astrocytes and glia, in response to stimulation by other cytokines like IL-1 and TNF α [325-328]. In vitro and in vivo studies have demonstrated the ability of IL-8 to increase vascular permeability in the presence of inflammatory leukocytes [327, 328] suggesting a potential role in the development of blood-brain barrier dysfunction.

Brain trauma induced IL-8 production was first described in 1994 by Ott's group when they demonstrated a rise in the serum IL-8 concentration soon after severe brain trauma [109]. In another study of 14 adults with severe TBI, Kossmann and colleague found that both the CSF and serum IL-8 levels were significantly elevated in the brain injured patients for up-to 21 days post injury, and the CSF IL-8 concentrations were significantly higher than those measured in the serum suggesting an intracranial production of IL-8 [125]. A positive association was demonstrated between the IL-8 levels and severe blood-brain barrier dysfunction, but IL-8 concentrations had no associations with the initial injury severity or neurological recovery [125]. Other investigators have also confirmed rises in serum and CSF IL-8 after brain trauma [126, 329], and the CSF IL-8 concentration may be associated with mortality of severe childhood brain injury [126].

5.1.2.1.3) Interleukin 10 (IL-10)

Interleukin 10 (IL-10) is a potent anti-inflammatory mediator with immunosuppressive actions. It has the ability to inhibit the synthesis of pro-inflammatory cytokines such as IL-1, TNF and adhesion molecules by macrophages [330-334], while at the same time, it promotes the production and secretion of their endogenous antagonists [335, 336]. In addition, IL-10 directly reverses the stimulating effects of these pro-inflammatory cytokines on glia [337, 338], macrophages and neutrophils [339].

Clinical studies of children and adults after traumatic brain injury have confirmed the presence of high level of IL-10 in both CSF and peripheral blood [122, 172, 175, 340]. The ventricular IL-10 concentration was first reported to be increased in 15 severely brain injured children in 1997 [122]. The elevated IL-10 level was noted to persist for 3 days following the initial injury and children less than 4 years of age appeared to have a higher concentration of IL-10 in the CSF [122]. This suggested an age dependent IL-10 production response following brain trauma. Furthermore, this study was also the first and only to report an association between the increased CSF IL-10 concentration and mortality [122].

Csuka's group confirmed elevation of IL-10 CSF concentrations after severe isolated brain injury among 28 adults [172]. They measured the paired CSF and serum IL-10 levels daily and found that the peak CSF IL-10 levels occurred on the first day post injury [172]. A smaller secondary rise was observed in the second week following trauma. The CSF level was higher than the corresponding serum concentration suggesting after brain damage IL-10 was produced intrathecally [172]. Neither the CSF nor serum IL-10 levels correlated to the functionality of the blood-brain barrier [172]. In another study of 29 adults with isolated TBI, Maier and co-workers similarly found no correlation between the blood-brain barrier dysfunction and IL-10 concentrations in plasma and CSF [175].

5.1.2.2) Adhesion Molecules

Soluble adhesion molecules belong to a family of less well investigated inflammatory mediators that have been found in high concentrations in the CSF following brain trauma. They include intercellular adhesion molecules (ICAM), vascular cellular adhesion molecules (VCAM), neural cell adhesion molecules (NCAM), E-selectin, L-selectin and P-selectin. The selectins are glycoproteins that mediate the initial rolling and early adhesion of leukocytes to the vessel wall by binding to endothelial receptors at the site of inflammation [341]. Deficiency of the selectins or their ligands would, therefore, result in failure of the leukocyte migration process and recurrent infections. Cellular adhesion molecules belong to the immunoglobulin superfamily. ICAM and VCAM are present on the endothelial surface while their counterreceptors, the integrins are found on the leukocyte surface. Through binding of the cellular adhesion molecules to their respective integrins, the process of strong adhesion and migration of leukocytes across the endothelium into tissue is mediated [341]. The selectins and cellular adhesion molecules are found in soluble active forms in the blood of normal humans but their precise function in healthy individuals remain unknown [342].

McKeating and colleagues from Edinburgh studied the systemic and jugular venous serum concentrations of ICAM-1 and sL-selectin, and their relationships with injury severity and outcome after adult TBI [169]. They found that although the admission serum ICAM-1 levels in head injured patients were similar to those measured in the controls, significant production of ICAM-1 was observed among

head injured patients within 96 hours of injury [169]. Furthermore, the serum SICAM-1 level was significantly related to both neurological recovery and injury severity as classified by the GCS [169]. The concentrations of sL-selectin in head injured patients, on the other hand, were consistently lower than those without brain trauma [169]. No transcranial gradient was found for either of the adhesion molecules suggesting that the changes in intracranial adhesion molecule activity may be the result of a systemic inflammatory response in addition to that produced intracranially [169].

An American study investigated the CSF concentrations of P-selectin, SICAM-1, E-selectin, L-selectin, and VCAM-1 in children with brain trauma or meningitis but found a heterogeneous expression pattern of the adhesion molecules among head injured children [170]. Some head injured children had increased CSF concentrations of the adhesion molecules similar to levels observed in children with meningitis while others did not show any increase CSF adhesion molecules post injury [170]. They also found that P-selectin was the only adhesion molecule that was modestly increased in the CSF at later times after TBI (12-24 hours and 24-48 hours) [170]. No previous study has examined the production of adhesion molecules beyond 3 days after brain trauma and their relationships with outcome prediction.

5.1.3) Vasoconstrictor (Endothelins)

Endothelins are peptides with potent vasoconstriction properties and have been reported to be present in higher concentration after various brain insults such as

spontaneous subarachnoid haemorrhage [343], brain trauma [343, 344], and neurosurgical procedures for epilepsy [344]. These case series suggested a potential correlation with neurological deterioration in patients with high CSF endothelin concentrations. A recent Chinese study investigated the clinical significance of early changes (within 24 hours of injury) in plasma endothelin (ET), nitric oxide (NO), and arginine-vasopressin (AVP) in adult brain injured patients [345]. They found that the plasma levels of these markers correlated with injury severity and patients with subdural haematoma had significantly higher concentration of ET, NO and AVP than those who had epidural haemorrhage [345]. Larger studies would be required to ascertain the role of endothelins and other vasoconstrictors following brain trauma and their relationships with outcome and injury severity prediction.

5.1.4) Difficulties in the Development of Neurochemical Monitoring

Although growing number of potential biomarkers of brain trauma continue to emerge in the literature, little progress has been made to successfully translate these research findings into clinically useful monitoring and prognostic technique. As demonstrated in the above section, some mediators have been investigated more extensively than others as potential markers of brain injury severity, recovery, and prognosticators. This is because majority of the research interests in the brain trauma – biomarker literature has so far concentrated on investigating individual mediators or a small number of mediators within the same biochemical family. It is unclear from the existing literature whether certain mediators better differentiate severe injury while others better predict outcome.

In medicine, combining several clinical features and tests results often provide more accurate diagnosis and prognostication than may be achieved with using the individual features or tests alone. It remains unknown whether this is also true for brain trauma outcome prediction using serum biomarkers.

5.2) AIMS

This chapter aims to determine and compare the predictive values of 8 different biochemical mediators' serum levels on injury severity and outcome after childhood brain trauma. We also aim to determine which mediator better identifies severe brain injury and predict poor outcome. Additionally, we aim to determine whether prognostic rules using 2 biomarker levels are better at predicting unfavourable outcome after childhood brain trauma than using individual marker level.

5.3) PATIENTS AND METHODS

Twenty-eight consecutive children (21 boys and 7 girls) requiring neuro-intensive care after isolated brain trauma were enrolled into a single-centre (Edinburgh RHSC) prospective observational study.

Clinical and demographic details were collected prospectively using a pre-designed proforma. Primary brain injury severity was assessed using the post-resuscitation/pre-intubation Glasgow Coma Scale (GCS), and was classified into

‘severe injury’ where GCS was 8 or less and ‘non-severe injury’ where GCS was more than 8.

Conscious level was assessed using the modified GCS at PICU discharge which equated to the end of neuro-intensive care when the brain injured children no longer required airway protection, ventilatory and circulatory supports. The children were dichotomised into those who had ‘regained consciousness’ (GCS > 8) and those with ‘delayed return of consciousness’ (GCS 8 or less).

Global outcome was assessed at 6 months post injury using the modified Glasgow Outcome Score (GOS) and divided into ‘good recovery’ when GOS 4 and 5 were achieved, and ‘unfavourable outcome’ when GOS were between 1 and 3.

5.3.1) Sample Collections

All patients had an arterial blood sample collected at 24 hours (day 1) post injury. Eighteen patients whose clinical condition required PICU stay beyond 5 days had an additional blood sample collected at 120 hours (day 5) post injury. The blood samples were collected in pyrogen-free plastic tubes and centrifuged at 1200 g for 10 minutes. The serum was removed immediately and stored in pyrogen-free plastic tubes at -70°C until analyses.

5.3.2) Markers Assays

Commercially available enzyme linked immunoassays (ELISA) were used to quantify the following serum biomarker concentrations:

- Glial Protein:
 - S-100B (Nexus Dx™ S-100 Test Kit, Synx Pharma Inc)
- Neuronal Protein:
 - NSE (Nexus Dx™ NSE Test Kit, Synx Pharma Inc)
- Interleukins:
 - IL-6 (IL-6 ELISA Kit, Diaclone Research)
 - IL-8 (IL-8 ELISA kit, Diaclone Research)
 - IL-10 (IL-10 ELISA Kit, Diaclone Research)
- Adhesion Molecules:
 - L-selectin (human sL-Selectin Immunoassay, R&D Systems)
 - SICAM (sICAM-1 ELISA Kit, Diaclone Research)
- Vasoconstrictor:
 - Endothelin (Endothelin 1-21 Test Kit, Biomedica)

Samples were analysed in duplicate and averaged to provide the marker concentration. I performed all biomarker ELISA for this study. There are many similarities between the different ELISA kits. To avoid being repetitive, the basic principles of ELISA is presented in the following section and details of the commercially available ELISA kits used in this study may be found in Appendix II.

5.3.2.1) Basic Principles of Sandwich Enzyme Linked Immuno-Sorbent Assays

Enzyme Linked Immuno-sorbent Assays (ELISA) are immunochemical methods for determination of substrates such as peptides, proteins, antibodies, and hormone, in which a crucial element of the detection is an antigen-antibody interaction. These assays utilize antibodies that are covalently linked to an enzyme which can catalyse an easily visualised reaction, such as colour change, when a substrate is added.

Biological samples containing the mediator to be tested are exposed to specific antibody to that particular mediator so that an antibody-antigen complex is formed. This antibody-antigen complex is detected by treating the test system with a conjugate i.e. another antibody linked to an enzyme to ensure formation of another antibody enzyme complex. This antibody enzyme complex serves as a marker and attaches only to a specific substrate. When a substrate for the enzyme is added to the assay, a reaction between the substrate and the conjugate is usually indicated by a colour change. If no mediator was present in the test sample to bind with the specific antibody, no conjugate interaction would take place and no colour change will occur at the time of detection.

Sandwich method ELISA forms the basic principle of the commercially available ELISA kits used in this study. As the name implies, the molecule tested is held between two different antibodies in this technique. Monoclonal antibody specific to the test molecule has been coated onto the wells of the microtiter strips provided in

the kit. Test samples, the supplied standardised amount of the test molecule, or control specimens are added to the wells and allowed to react with the bound antibody. After washing, a known amount of enzyme-labelled antibody is added and allowed to react with the bound test molecule. Any excess unbound enzyme-linked antibody is washed away after the reaction. The substrate is added and the reaction between the substrate and the enzyme produces a colour change. The amount of visual colour change is a direct measurement of the specific enzyme-conjugated bound antibody, and is proportional to the concentration of the test molecule present in the samples. The colour change is assessed using a fibre-optic multichannel spectrometer.

5.3.3) Statistical Analysis

Non-parametric statistical tests were used and data were given as median and interquartile range because the observed mediator levels did not follow a normal distribution. Mann Whitney U tests were employed to detect statistical difference in mediator concentrations between dichotomies for injury severity, PICU discharge coma status, and outcome at 6 months. For each mediator that had significantly different levels between each assessment dichotomies, sensitivity and specificity of incremental values as predictor of severe injury, coma at PICU discharge, and unfavourable outcome at 6 months were calculated.

5.3.3.1) Receiver-Operator Characteristic (ROC) Curve Analysis

Receiver Operator Characteristic (ROC) curves were then plotted and the optimal cut-off value for each mediator, which was defined as the point closest to the left upper corner of the ROC curve, was identified. Areas under the ROC curves were measured to compare the predictive values between mediators.

5.3.3.2) Multivariate Receiver-Operator Characteristic (MultiROC) Curve Analyses

Multivariate Receiver-Operator Characteristic (MultiROC) curves analysis was used to assess the outcome predictability of the pre-defined prognostic rules that had specific criteria for the serum levels of 2 biomarkers. One of the 2 biomarkers within each prognostic rule acted as ‘the screening marker’ and was fixed at the prognostic threshold while the other biomarker acted as the ‘varying marker’ so that corresponding sensitivities and specificities at incremental values were calculated to plot the MultiROC curve.

5.3.3.2.1) Defining Prognostic Thresholds for Screening Markers within the Prognostic Rules

To define the prognostic threshold for each of the ‘screening marker’ within the prognostic rules, each marker level was plotted against the outcome dichotomies and the level which identified the most patients with good recovery without including

any patient with unfavourable outcome was used as the prognostic threshold for that particular marker. The prognostic thresholds for each of the 8 biomarkers were summarised in Table 5.1.

Table 5.1:

	Prognostic Threshold
Glial Protein: S100B	0.04 ng/ml
Neuronal Protein: NSE	12 ng/ml
Interleukins: IL-6	30 pg/ml
IL-8	20 pg/ml
IL-10	Level did not differentiate outcome
Adhesion Molecules: SICAM	700 ng/ml
L-Selectin	700 ng/ml
Vasoconstrictor: Endothelin	Level did not differentiate outcome

5.3.3.2.2) Defining Prognostic Rules for MultiROC Analyses

Because IL-10 and endothelin levels did not differentiate between the outcome dichotomies, they were excluded from MultiROC analyses. There were, therefore, 6 possible screening markers, and each screening marker was paired in turn with the remaining 5 biomarkers to form 30 different prognostic rules which were summarised in Table 5.2.

Table 5.2:

Prognostic Rules for MultiROC Analyses
IL-6 ≥ 30 pg/ml as the screening marker: <ul style="list-style-type: none"> • IL-6 ≥ 30 pg/ml AND IL-8 at varying incremental cutpoints • IL-6 ≥ 30 pg/ml AND S100B at varying incremental cutpoints • IL-6 ≥ 30 pg/ml AND NSE at varying incremental cutpoints • IL-6 ≥ 30 pg/ml AND SICAM at varying incremental cutpoints • IL-6 ≥ 30 pg/ml AND L-Selectin at varying incremental cutpoints
IL-8 ≥ 20 pg/ml as the screening marker: <ul style="list-style-type: none"> • IL-8 ≥ 20 pg/ml AND IL-6 at varying incremental cutpoints • IL-8 ≥ 20 pg/ml AND S100B at varying incremental cutpoints • IL-8 ≥ 20 pg/ml AND NSE at varying incremental cutpoints • IL-8 ≥ 20 pg/ml AND SICAM at varying incremental cutpoints • IL-8 ≥ 20 pg/ml AND L-Selectin at varying incremental cutpoints
L-Selectin ≥ 700 ng/ml as the screening marker: <ul style="list-style-type: none"> • L-Selectin ≥ 700 ng/ml AND IL-6 at varying incremental cutpoints • L-Selectin ≥ 700 ng/ml AND IL-8 at varying incremental cutpoints • L-Selectin ≥ 700 ng/ml AND S-100B at varying incremental cutpoints • L-Selectin ≥ 700 ng/ml AND NSE at varying incremental cutpoints • L-Selectin ≥ 700 ng/ml AND SICAM at varying incremental cutpoints
SICAM ≥ 700 ng/ml as the screening marker: <ul style="list-style-type: none"> • SICAM ≥ 700 ng/ml AND IL-6 at varying incremental cutpoints • SICAM ≥ 700 ng/ml AND IL-8 at varying incremental cutpoints • SICAM ≥ 700 ng/ml AND S100B at varying incremental cutpoints • SICAM ≥ 700 ng/ml AND NSE at varying incremental cutpoints • SICAM ≥ 700 ng/ml AND L-Selectin at varying incremental cutpoints
S100B ≥ 0.04 ng/ml as the screening marker: <ul style="list-style-type: none"> • S100B ≥ 0.04 ng/ml AND IL-6 at varying incremental cutpoints • S100B ≥ 0.04 ng/ml AND IL-8 at varying incremental cutpoints • S100B ≥ 0.04 ng/ml AND NSE at varying incremental cutpoints • S100B ≥ 0.04 ng/ml AND SICAM at varying incremental cutpoints • S100B ≥ 0.04 ng/ml AND L-Selectin at varying incremental cutpoints
NSE ≥ 12 ng/ml as the screening marker: <ul style="list-style-type: none"> • NSE ≥ 12 ng/ml AND IL-6 at varying incremental cutpoints • NSE ≥ 12 ng/ml AND IL-8 at varying incremental cutpoints • NSE ≥ 12 ng/ml AND S100B at varying incremental cutpoints • NSE ≥ 12 ng/ml AND SICAM at varying incremental cutpoints • NSE ≥ 12 ng/ml AND L-Selectin at varying incremental cutpoints

5.3.3.2.3) Assessment of the Predictive Values of the Prognostic Rules

Areas under the MultiROC curves (AUC) were measured to compare the predictive values between the different prognostic rules. The predictive values of the prognostic rules were also compared with the predictive value of each individual

mediator of the same cohort to determine whether combining 2 biomarker levels had a more superior outcome prognostic value. The optimal cut-off value for each of the varying mediator within the prognostic rules, which was defined as the point closest to the left upper corner of the MultiROC curve, was also identified.

5.4) RESULTS

5.4.1) Injury severity and Outcome

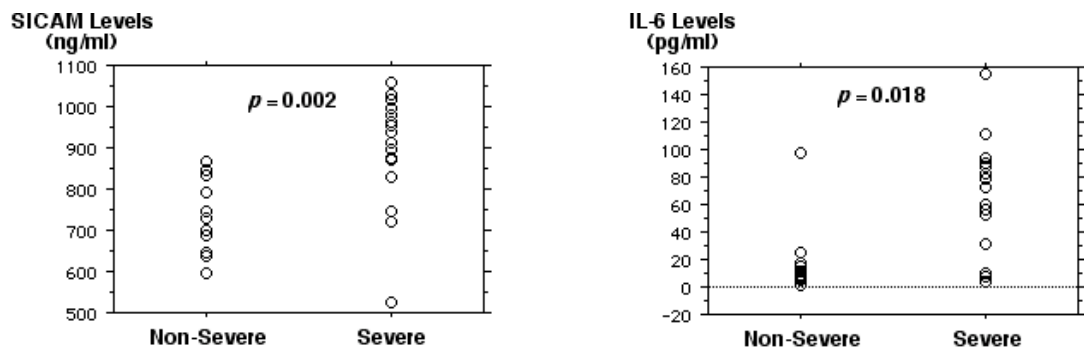
Of the 28 children included in the study, 17 had severe injury while 11 had non-severe injury. 19 patients had diffuse brain injury demonstrated on the initial CT brain scan. At PICU discharge, 23 patients regained consciousness while 5 remained in a coma. At 6 months post injury, 24 patients made a good recovery while 4 children had an unfavourable outcome of whom only 1 had regained consciousness at PICU discharge ($p = 0.01$, Fisher's Exact Test).

In the subgroup of 18 patients with day 5 mediator concentrations measured, 14 had severe injury. 13 regained consciousness and 5 remained in a coma at PICU discharge. 4 children had unfavourable outcome at 6 months of whom 3 had remained in a coma at PICU discharge ($p = 0.04$, Fisher's Exact Test).

5.4.2) Mediator Concentrations and Injury Severity

Children with severe injury had significantly higher day 1 serum concentrations of SICAM ($p = 0.002$) and IL-6 ($p = 0.018$) than those with non-severe injury (Figure 5.1). The area under the ROC curve (AUC) for SICAM was 0.834 while that for IL-6 was 0.791 indicating that SICAM was more superior in the diagnosis of severe injury than IL-6 (Table 5.3). No difference was found in any of the day 5 serum marker concentrations between patients with different injury severity.

Figure 5.1:



5.4.3) Mediator Concentrations and Initial Brain CT Findings

NSE day 1 serum level was the only mediator level that was significantly raised in children with diffuse injury demonstrated on the initial brain CT scan when compared to those with focal injury (median levels for diffuse and focal injury were 15.21 and 7.31 ng/ml respectively, $p = 0.01$, Mann Whitney U test; AUC = 0.792).

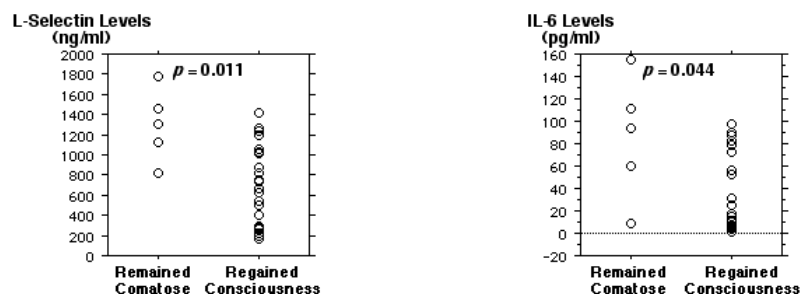
5.4.4) Mediator Concentrations and Outcome

5.4.4.1) At PICU Discharge

The day 1 IL-6 and L-selectin serum levels were significantly higher among patients who remained comatose at PICU discharge than those who had regained consciousness (Figure 5.2). This was true for the day 5 serum level of S100B, NSE and L-selectin (Figure 5.2). Although day 1 L-selectin level had a better predictive value than IL-6 for coma status at PICU discharge (Table 5.3), S100B day 5 level had the best predictability of the PICU discharge coma status with a sensitivity of 80% and a specificity of 85% at the optimal cut-off level of 0.025 ng/ml (Table 5.3).

Figure 5.2:

Day 1 Marker Levels & PICU Discharge Conscious State



Day 5 Marker Levels & PICU Discharge Conscious State

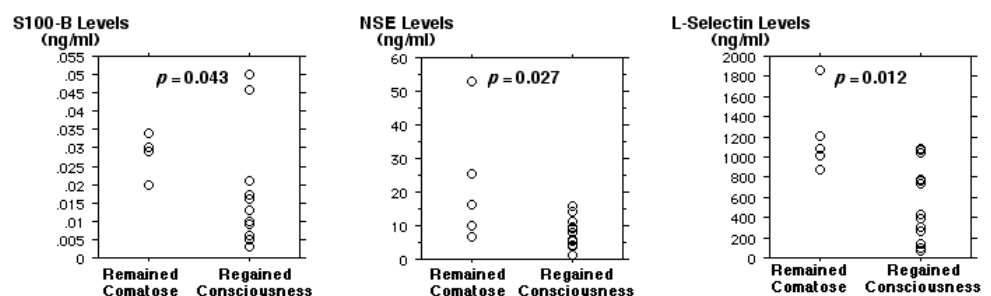


Table 5.3:

Indication of Severe Injury				
	Optimal Cut-off Value on ROC	Sensitivity	Specificity	AUC
SICAM Day 1 Level	800 ng/ml	76%	73%	0.83
IL-6 Day 1 Level	40 pg/ml	61%	91%	0.79
Prediction of Coma at PICU Discharge				
	Optimal Cut-off Value on ROC	Sensitivity	Specificity	AUC
S100B Day 5 Level	0.025 ng/ml	80%	85%	0.88
NSE Day 5 Level	10 ng/ml	80%	77%	0.87
L-selectin Day 5 Level	800 ng/ml	100%	77%	0.86
L-selectin Day 1 Level	1000 ng/ml	80%	70%	0.86
IL-6 Day 1 Level	40 pg/ml	80%	61%	0.78
Prediction of Poor Outcome at 6 Months Post Injury				
	Optimal Cut-off Value on ROC	Sensitivity	Specificity	AUC
L-selectin Day 1 Level	1200 ng/ml	75%	88%	0.92
L-selectin Day 5 Level	800 ng/ml	100%	71%	0.89
IL-8 Day 1 Level	30 pg/ml	75%	92%	0.88
NSE Day 1 Level	25 ng/ml	75%	83%	0.83
S100B Day 1 Level	0.05 ng/ml	75%	79%	0.83
IL-6 Day 1 Level	60 pg/ml	75%	71%	0.83
NSE Day 5 Level	10 ng/ml	75%	71%	0.82

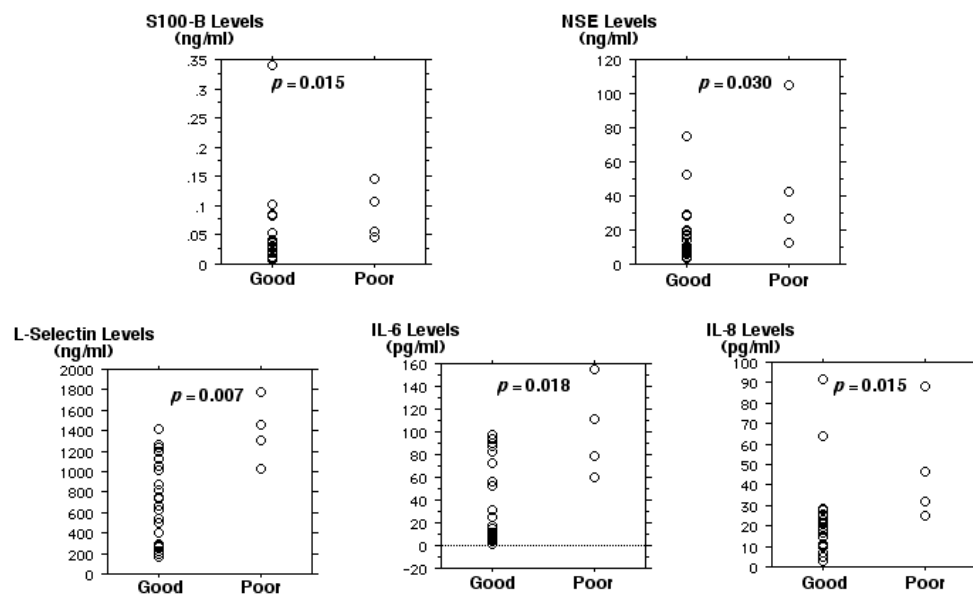
5.4.4.2) 6 Months Post Injury

Patients with unfavourable outcome had significantly higher day 1 serum concentrations of S-100B, NSE, L-selectin, IL-6, and IL-8 than those with good recovery (Figure 5.3). When considering the day 5 marker serum levels, only L-selectin and NSE levels were significantly higher in children with unfavourable outcome than those with good recovery (Figure 5.3). Day 1 L-selectin level had the

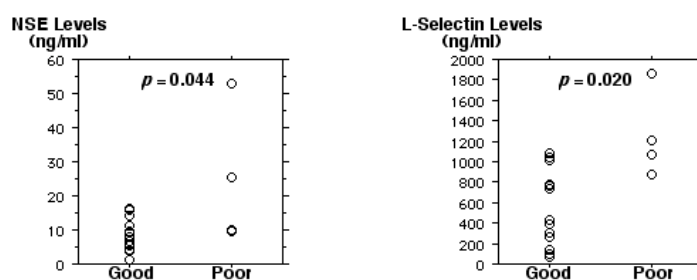
best predictive value for unfavourable outcome at 6 months after brain trauma with a sensitivity of 75% and a specificity of 88% at the optimal cut-off value of 1200 ng/ml (Table 5.3).

Figure 5.3:

Day 1 Marker Levels & 6-Month Outcome



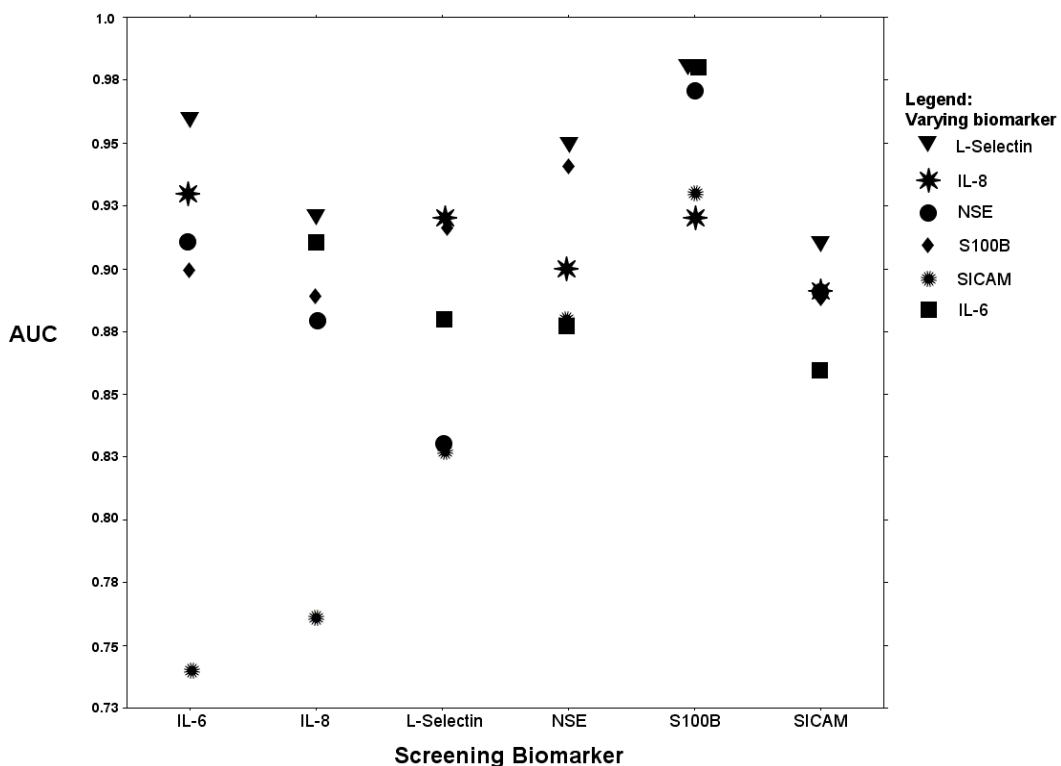
Day 5 Marker Levels & 6-Month Outcome



5.4.5) Comparisons of the Outcome Prediction Performance Between the Different Prognostic Rules and Individual Marker Levels

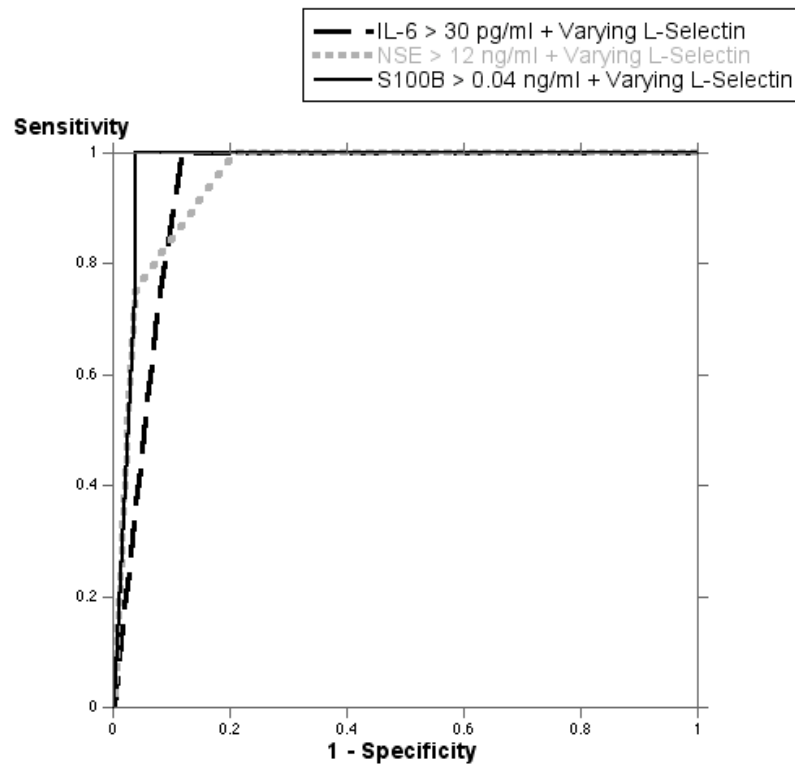
When using individual marker level to predict unfavourable outcome, only the day 1 L-selectin level had an AUC > 0.9. Of the 30 prognostic rules examined using combination of 2 biomarker levels, 17 had AUC > 0.9 for prediction of unfavourable outcome after brain trauma (Figure 5.4). Prognostic rules using S100B > 0.04 ng/ml as the screening markers had better predictive values for unfavourable outcome than rules using other markers as the screening marker (Figure 5.4). L-selectin when used as the varying marker within the prognostic rules had better predictive values for unfavourable outcome than when used as the screening marker within the prognostic rules (Figure 5.4).

Figure 5.4:



As described above, Day 1 L-Selectin level of 1200ng/ml was 75% sensitive and 88% specific for predicting poor outcome after brain trauma. When considering the prognostic rule that used the same L-Selectin level cut point (i.e. at 75% sensitivity) but combined with S100B as the screening marker, the specificity increased to 96% for prediction of unfavourable outcome (Figure 5.5). Similarly if NSE > 12 ng/ml was used as the screening marker with L-selectin day 1 level of 1200 ng/ml in the prognostic rule, the sensitivity was 75% and specificity was 96% for predicting unfavourable outcome (Figure 5.5). The improvement in specificity was less if L-selectin was paired with an inflammatory marker in the prognostic rule and only increased to 92% when paired with IL-6 as the screening marker (Figure 5.5).

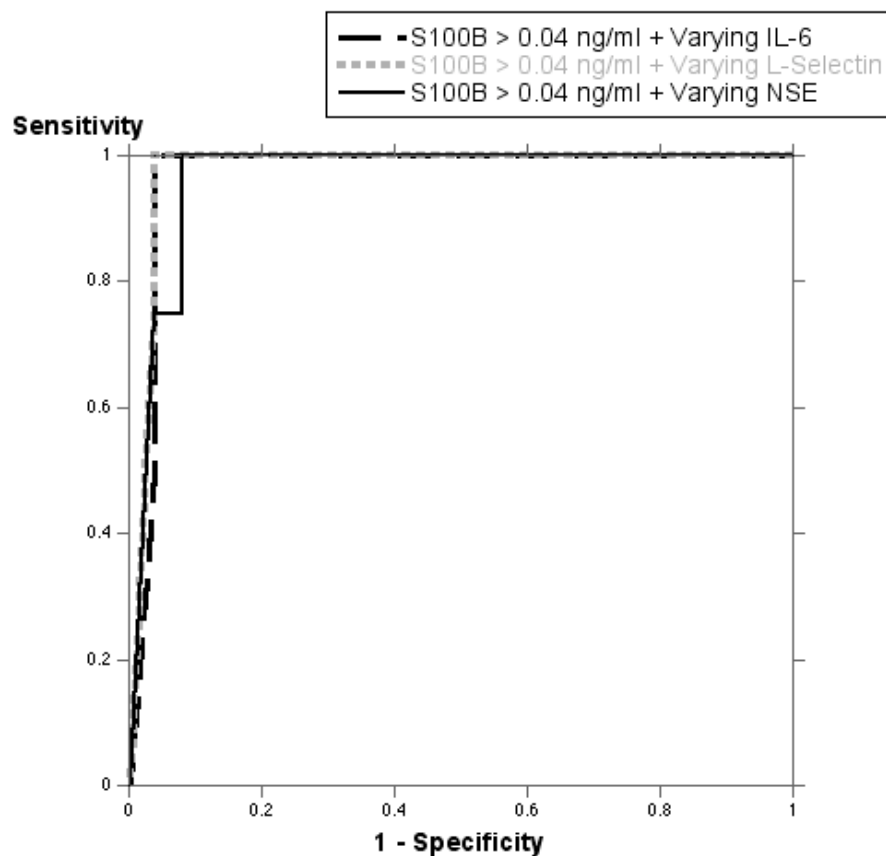
Figure 5.5



The following prognostic rules were 100% sensitivity and 96% specific for predicting unfavourable outcome after childhood brain trauma in our cohort (Figure 5.6):

1. Day 1 serum S100B level > 0.04 ng/ml AND Day 1 L-selectin level of 1000 ng/ml or more. (AUC 0.98)
2. Day 1 serum S100B level > 0.04 ng/ml AND Day 1 IL-6 serum level of 40 pg/ml or more. (AUC 0.98)

Figure 5.6:



5.5) DISCUSSIONS

This study simultaneously evaluated the serum concentrations of multiple biomarkers of different mediator families and their predictive values for severe injury and unfavourable outcome in an exclusive child cohort measured at two distinct time points after brain trauma. We found that adhesion molecules had better predictive values for severe injury and poor outcome after brain trauma than glial or neuronal proteins, while glial protein had the best predictive value for PICU discharge coma status. In addition, we demonstrated prognostic rules combining 2 biomarker levels had more superior outcome prediction than could be achieved using individual marker levels.

5.5.1) Investigating the Diagnostic and Predictive Values of Multiple Biomarkers of Different Mediator Families

Majority of the reports in the current brain trauma biomarker literature have focused on investigating individual mediators or a small number of mediators of the same biochemical family [119, 121-123, 125, 126, 163-167, 169-172, 267, 309, 317, 346-348]. Furthermore, they used different sample types and sampling time points after injury making comparisons between study results difficult. Our study, in contrast, simultaneously evaluated the serum levels of 8 biomarkers which were chosen from the main mediator families of previously reported potential brain trauma markers namely: (i) glial proteins (S100B), (ii) neuronal proteins (NSE); (iii) interleukins (IL-6, IL-8, and IL-10), (iv) adhesion molecules (SICAM and L-selectin); and (v)

vasoconstrictors (endothelins). This allowed us to investigate the diagnostic and prognostic abilities of some of the previously lesser investigated biomarkers such as adhesion molecules in addition to the more frequently studied biomarkers like S100B and NSE.

Receiver-Operator Characteristic (ROC) curve analysis was used to compare these markers' diagnostic and outcome predictive values because it is a powerful tool for evaluating a medical test's validity without the need to define one single arbitrary cut-off level [205]. It provides a simple and easily interpretable graphical display of the trade-off between sensitivity and specificity at each decision thresholds [205]. Furthermore, calculating and comparing the area under the ROC curves (AUC) of the different tests assess predictive discrimination with an AUC of 1.0 equating to perfect prediction while that of 0.5 indicating random guessing [349]. Using this simple but powerful tool, we were, therefore, able to report for the first time that out of the 8 biomarkers evaluated, SICAM was the best severe brain injury indicator while L-selectin had the best predictability of unfavourable outcome after childhood brain trauma.

5.5.2) Biomarkers and Indication of Injury Severity

IL-6 [123, 350-352], SICAM [169], S100B [353, 354], and NSE [163] have independently been associated with severe brain injury in previous adult and paediatric brain trauma studies. IL-6 is able to cause and inhibit inflammation after brain injury. Its serum and CSF levels measured at various time points up to 12

hours after brain trauma have been extensively reported to differentiate injury severity in adults and children [123, 350, 352, 355, 356]. Our finding is consistent with these previous reports [123, 350, 352, 355, 356] and suggested that IL-6 level measured at 24 hours post injury was still useful in identifying severe brain trauma in children.

SICAM is present in soluble active forms in the blood of normal humans but its precise function in healthy individuals remains unknown. Although it is less well investigated previously, CSF and arterial serum SICAM levels have been shown to correlate with injury severity in adults when measured at 24 hours after brain trauma [169, 347]. Our finding is, therefore, consistent with these previous reports. Given the limited knowledge on the function of SICAM post brain trauma, it is difficult to ascertain why it is potentially more superior in diagnosing severe brain injury than other markers in our cohort and warrants further investigations.

L-selectin is another adhesion molecule that has been previously reported to differentiate injury severity after brain trauma. Similar to SICAM, little information is available on its function in humans. Its serum level measured after brain trauma has been found to be persistently below those detectable in normal healthy adults [169]. McKeating and colleague additionally found that when considering its concentrations in brain trauma adults, those who sustained severe brain trauma had significantly higher serum L-selectin levels than those with non-severe injury [169]. We did not demonstrate any association between L-selectin level and injury severity

in our exclusive child cohort. Further studies are necessary to ascertain its relationship with injury severity after brain trauma.

S100B is a calcium binding protein in astrocytes and NSE is a soluble cytoplasmic protein localised principally in neurons and neuroendocrine cells. Although raised serum levels of these glial and neuronal proteins indicate irreversible astrocyte and neuronal injury, their ability to quantify brain injury severity remains controversial with only limited reports describing an association between severe injury and early serum levels measured in the emergency department or on admission to ICU [163, 353, 354]. In our cohort, S100B and NSE serum levels did not differentiate injury severity. This was consistent with previous reports where serum measurement of these markers at 24 hours post injury failed to relate to severe brain trauma [270, 357]. This suggested that the timing of the assay after the initial injury was particularly important if glial or neuronal proteins were used to diagnose severe brain trauma and this may be related to their relatively short serum half-life.

5.5.3) Biomarkers and Prediction of PICU Discharge Coma Status

In our cohort, only 1 of the 23 patients who regained consciousness at PICU discharge had unfavourable outcome at 6 months after brain trauma. This reaffirmed the relationship between conscious state and outcome after brain trauma. We found that brain specific proteins measured on the fifth day post injury had the best predictive value for PICU discharge coma status even though their levels on day 1 had no association with the conscious state at the completion of neuro-intensive care.

It is possible that day 5 marker levels represent the total burden of brain damage from both primary brain injury and second insults and may, therefore, better differentiate patients who are likely to remain comatose from those who will regain consciousness at PICU discharge. Reactive astrogliosis has previously been reported to associate with a secondary rise in S100B levels after brain trauma [311] and this may explain our finding. Alternatively, the rate of recovery in conscious levels may vary with the speed of decline in the serum levels of glial or neuronal proteins. Further studies are required to assess this postulate and to determine whether the decline rate of these markers' serum levels may potentially be used as a monitor of clinical recovery.

5.5.4) Biomarkers and Outcome Prediction

5.5.4.1) Brain Trauma Outcome Prediction with Glial and Neuronal Proteins

Brain specific proteins are the most frequently investigated potential brain trauma biomarkers, and many studies have reported an association between poor outcome and elevated serum levels of glial and neuronal proteins in adults and children. In a study of 41 head injured adults with varying degree of severity, Rothoerl and colleague found that the mean serum S-100B levels were significantly higher in those with unfavourable outcome [168]. Jackson and co-workers examined the admission and 4-hour post injury serum S100B levels of 30 severely brain injured adults and found that only the admission serum level correlated with outcome [316].

In another study involving 84 patients with an admission GCS ≤ 8 , the peak serum S-100B levels were significantly higher among patients with unfavourable outcome when compared to those with favourable recovery, but the serum NSE levels did not differ between the two groups [317]. Evaluation of the serum S100B levels of 27 children measured within 12 hours of sustaining brain trauma showed that children with poor outcome (Pediatric Cerebral Performance Category score of less than 4) at 6 months post injury had significantly higher S100B concentrations than those with good recovery [358]. In another childhood brain trauma study, the emergency department admission serum NSE levels of the 86 patients predicted poor outcome at the time of hospital discharge with 74% specificity and 86% sensitivity using a cut-off value of 21.2ng/ml [359]. However, others investigators had also failed to demonstrate any correlation between the serum or CSF NSE concentrations and neurological recovery [166, 310]. Our finding with S100B and NSE day 1 levels and poor outcome was, therefore, consistent with these previous reports.

5.5.4.2) Brain Trauma Outcome Prediction with Interleukins

Inflammatory mediators have similarly been reported to have potential outcome predictive value after brain trauma. In a study of 94 head injured adults, the maximum IL-6 plasma levels in fatal cases were 8 times higher than those measured in the survivors [360]. In another study of 22 patients with severe brain trauma, serum IL-6 and IL-8 levels measured after 72 hours post injury were significantly higher in the fatal group [176]. Minambres and colleagues assessed paired arterial and venous serum IL-6 levels in 62 adults requiring intensive care after brain trauma

and found that elevated transcranial gradient of IL-6 was associated with poor outcome at 6 month post injury [352]. Our study confirmed that serum IL-6 and IL-8 levels were predictive of unfavourable outcome after childhood brain trauma. The outcome predictive value of IL-6 in our cohort was inferior to that of IL-8, while IL-10 had no association with outcome. This indicated that mediators from the same biochemical family may have very different outcome predictability after brain trauma and highlighted the need to study multiple markers even if they belonged to the same biochemical family.

5.5.4.3) Brain Trauma Outcome Prediction with Adhesion Molecules

In addition to IL-6 and IL-8, we found L-selectin serum levels to be highly predictive of unfavourable outcome after childhood brain trauma. It is one of the least investigated potential biomarkers of brain trauma and, to the best of my knowledge, our study is the first to describe its association with poor outcome after brain trauma. Further studies are, therefore, warranted to investigate this promising brain trauma biomarker.

5.5.4.4) L-Selectin and IL-8 Serum Levels had the Highest Outcome Predictive Values

It is unclear from the exiting literature whether acute serum levels of brain specific proteins or inflammatory mediators have more accurate brain trauma outcome prediction. This is because majority of these previous studies concentrated on

evaluating a single mediator or limited numbers of mediators from the same biomarker family. Additionally, the reports in the literature often use different specimen types, sample collection time points, and data analyses making comparisons between studies difficult. Our study demonstrated that some inflammatory markers (L-selectin and IL-8) had better predictability of unfavourable outcome after brain trauma than glial or neuronal proteins when measured on day 1 and 5 post injury. The relatively short serum half-life of glial and neuronal proteins may explain this observation and serum levels of these proteins measured sooner after brain trauma may offer more superior outcome prediction. Another possible interpretation is that although serum levels of glial and neuronal proteins estimate astrocyte and neuronal damage, they are unable to accurately quantify the degree of damage. Serum inflammatory marker levels may, on the other hand, have better associations with the actual burden of neuro-inflammation. Alternatively, neuro-inflammation may have greater influence on outcome after brain trauma than previously expected.

We also demonstrated that the day 1 L-selectin level had a higher predictive value for unfavourable outcome than its level measured on the fifth day post injury, and neither IL-6 nor IL-8 day 5 levels were associated with poor outcome. Different marker's decline time course may explain this observation. Neuro-inflammation is a heterogeneous process with different pathways operating and subsiding at various time points after brain trauma. It is, therefore, important to further evaluate the pathways involved in neuro-inflammation and their time course after brain trauma.

5.5.4.5) Brain Trauma Outcome Prediction with Prognostic Rules Combining 2 Biomarker Levels

Brain trauma outcome is dependent upon many factors, and to expect successful and accurate prognostication with a single serum biomarker is unrealistic. However, most previous studies of serum biomarkers for brain trauma only investigated the relationships between individual markers and outcome. Other investigators who simultaneously assessed a small number of mediators from the same biomarker family compared each marker's outcome predictive values without considering combined markers' predictability. To the best of my knowledge, this study is the first to report the usefulness of prognostic rules combining 2 biomarker levels on outcome prediction after brain trauma.

5.5.4.5.1) Multivariate Receiver-Operator Characteristic (MultiROC) Curve Analysis

Powerful statistical techniques such as linear discriminate analysis and logistic regression are able to combine the results of multiple tests to form decision trees, but require expert statistical assistance. In addition, the derived diagnostic or prognostic rules are often too complex to apply to day-to-day clinical practice and are, therefore, restricted to research use only. Although ROC curves are simple to interpret and allowed robust assessment of a medical test's validity, they are limited to the display of a single test's performance, or the comparison of different single tests. Shultz described a novel multivariate extension to ROC curve analysis that allowed

comparisons between the performances of multivariate rules while retaining the simplicity of interpretation of the traditional ROC curves [361].

To construct a multivariate ROC (MultiROC) curve, a pre-defined diagnostic rule using two or more test results is required [361]. The pre-defined diagnostic rule will typically consist of a Boolean expression (i.e. containing ‘and’, ‘or’, ‘not’) of different tests related by algebraic operators (addition, subtraction, multiplication, division, equivalence, less than, or greater than) [361]. Each component of the rule is fixed at a specific diagnostic threshold with the exception of one component which is varied over all of its possible values, and the corresponding sensitivity and specificity are calculated to create the curve [361]. Interpretation of the MultiROC curve is the same as the traditional ROC curve [361]. MultiROC curves may, therefore, be created for different diagnostic rules to compare their diagnostic accuracy using the graphical displays [361]. Alternatively, the diagnostic performance between different rules may be assessed using the calculated areas under the MultiROC curves [361].

5.5.4.5.2) Screening and Varying Markers in Prognostic Rules and Their Effects on the Rules’ Prognostic Performance

Utilising the MultiROC curve analysis, we demonstrated that prognostic rules combining 2 biomarkers had a higher prognostic accuracy for outcome prediction than using individual marker’s levels after brain trauma. In addition, we found that the rules’ performance varied greatly with the choice of the screening and varying

markers within the prognostic rule. Prognostic rules that used a glial or neuronal protein as the screening marker had better predictability than rules that used either of these proteins as the varying marker. On the other hand, prognostic rules that used L-selectin as the varying marker offered more superior outcome prediction than those using it as the screening marker. One possible explanation to these observations was that the screening marker thresholds we chose for the glial or neuronal markers were more sensitive in screening for patients with unfavourable outcome than that chosen for L-selectin. Alternatively, although elevated glial and neuronal proteins signalled irreversible glial and neuronal damages, their relatively short serum half lives meant that their serum levels at the time of sampling in our cohort were better served as a screening tool for patients with unfavourable outcome.

We also found that outcome prediction with prognostic rules that used 2 biomarkers from the same mediator family was inferior to rules that utilized 2 markers from different mediator families. The most superior prediction of unfavourable outcome was achieved with rules that utilised S100B as the screening marker and either L-selectin (an adhesion molecule) or IL-6 (an interleukin) as the varying marker. These rules were 100% sensitive and 96% specific in their prognostic accuracy. The enhanced performance observed with these rules may be because of their ability to draw information from the degree of glial damage and neuroinflammation. This may also explain why prognostic rules that combined interleukins and SICAM had very inferior predictive values as they only assessed the degree of inflammation without considering glial or neuronal damage.

5.5.5) Limitations of the Study

The major limitation of our study is the small cohort size and paucity of patients with unfavourable outcome resulting in the need to assess outcome in dichotomies. In addition, because of the small cohort size, we were not able to validate the proposed prognostic rules.

Brain trauma induced cellular and molecular pathophysiological processes are modulated by many more mediators than the 8 biomarkers we chose for evaluation in this study. Although it is a step forward from investigating individual markers, we have only investigated the outcome predictive values of 8 different biomarkers and prognostic rules that combined 2 of these chosen biomarkers at any one time. Predictability of mediators not studied in this study requires further investigation and may have more superior outcome prediction than our chosen biomarkers.

5.6) CONCLUSIONS

Adhesion molecules may be more useful in diagnosing severe injury and predicting poor outcome after childhood brain trauma than brain specific proteins.

Combining 2 biomarker levels in prognostic rules offered more superior outcome prediction than using the individual marker's levels. Prognostic rules that utilize the day 1 serum levels of 2 biomarkers from different mediator families may predict unfavourable outcome after brain trauma with 100% sensitivity and 96% specificity.

A larger clinical study is required to validate these proposed prognostic rules and to assess their relationships with CPP insult.

CHAPTER 6: NEUROPATHOLOGICAL FEATURES OF FATAL PAEDIATRIC TBI

6.1) BACKGROUND

Brain trauma remains the commonest cause of childhood death in Britain despite significant advances in resuscitation skills and trauma care over the past two decades. It has been estimated that fatal outcome occurs between 5 to 20% of all acute childhood brain trauma requiring hospital admissions [1, 145, 146]. In spite of its frequent occurrence, only a few studies in the literature have detailed the pathological findings of fatal childhood brain trauma.

Between the 1960s and 1980s, a number of historic reports described the types of brain damage encountered among fatal non-missile head injury in adults and children [362-368]. However, comparisons of these studies were difficult because their findings relied upon data originating from two distinct types of studies: some studies consisted of only macroscopic examinations of large number of brain trauma fatalities but failed to recognise many important brain damage features, while the others consisted of small series of detailed neuropathological studies which were too selective to be of much general value.

In 1980, Adams and colleagues utilized a unique systematic post-mortem reporting method to document the detailed neuropathological features of 151 cases of fatal non-missile brain injury [369]. They demonstrated that important severe brain

damage features might be missed at necropsy unless various lesions were looked for specifically [369]. In addition, they introduced a novel quantitative contusion index which allowed for the first time a statistical approach to the assessment of contusions [369]. Both the systematic post-mortem reporting method and the quantitative contusion index have since been adopted by other investigators to summarise neuropathological features of fatal brain trauma in both adult and childhood populations.

Adams' cohort included both adult and childhood brain trauma fatalities. The first comprehensive report of detailed neuropathological features of fatal paediatric brain trauma appeared in the literature in 1989 when Graham and co-workers used the same systematic reporting system as Adams' report to document necropsy features of 87 fatal childhood accidental brain trauma from the west of Scotland [60]. Their cohort comprised of fatal brain trauma patients aged between 2 and 15 years identified from their main cohort of 635 cases of accidental brain trauma occurring between 1968 and 1982 [60]. They found that the pattern of brain damage observed in fatal paediatric brain trauma was generally similar to those identified in adult brain trauma fatalities [60]. This was not a surprising finding given their cohort excluded infants and children younger than 2 years of age based upon the known developmental difference in the skull base and calvaria in this age group compared with those of adults. The only significant difference they found between paediatric and adult brain trauma fatalities was the prevalence of diffuse brain swelling which occurred 4 times more frequently in children than in adults [60]. This finding

supported other neuroimaging reports where acute brain swelling was a frequent finding among comatose children following brain trauma [370].

Hypoxic ischaemic brain damage is another frequent post-mortem finding of fatal brain trauma and was identified in 61% of the paediatric brain trauma fatalities reported by Graham's group [60] when resuscitation skills and trauma care were not as vigilant as the current system in place in Scotland. It is unknown whether the improvement in acute trauma care training and provision over the past 2 decades has any effect on the prevalence of hypoxic ischaemic brain damage identified at post mortem examinations. Furthermore, no previous study has investigated the correlation between the amount of cerebral perfusion pressure insult experienced by paediatric brain trauma patients prior to death and the degree of hypoxic ischaemic brain damage identified at post-mortem.

Advancement in neuro-imaging and histopathological examination techniques enables white matter damage or axonal injuries to be identified more frequently in brain trauma patients. The introduction and routine application of immunohistochemical staining for β -APP have greatly facilitated the assessment of axonal damage in fatal brain trauma [371-373]. This antibody can be used to identify areas of disrupted axonal flow. Although it is only a marker of axonal damage and is not specific for trauma, it is still useful in the assessment of diffuse traumatic axonal injury (TAI). Damaged axons in adults have been identified among those who survived for as little as 35 minutes following the initial brain trauma using this technique [374], and the immunoreactivity is maximal at about 24

hours post-injury [373]. However, the time course and distribution of traumatic axonal pathology in infants and children has never been adequately studied, and there are good reasons to anticipate differences due to the varying degree of myelination with age and the vulnerability of the developing brain to metabolic insults such as hypoxia [375] and hypoglycaemia [376] which have been postulated as an important mechanism of secondary axonal injury.

Many advances have been introduced into modern trauma care over the past twenty years. These changes included improvement in resuscitation training and its provision, early transfer of brain trauma patients to trauma centres, and early instigation of neuro-intensive care. However, little is known on how these clinical management changes have affected neuropathological features of fatal childhood brain trauma.

Some neuropathological features of fatal brain trauma such as diffuse axonal injury and hypoxic ischaemic brain damage have been accepted to require time to evolve clinically and most published neuropathological series of fatal brain trauma have only examined fatalities that have survived long enough to reach neurosurgical centres and excluded instantaneous brain trauma deaths. This latter group of patients are unique given their primary mechanical brain injury was so severe that death occurred instantaneously or very shortly after the injury was sustained, and therefore, secondary brain damage should contribute very little towards its brain damage pattern seen at post mortem. It remains unknown whether diffuse traumatic axonal injury or hypoxic ischaemic brain damage is also found in instantaneous deaths.

6.2) HYPOTHESES AND AIMS

We hypothesize that (i) advances in modern trauma care over the past twenty years to minimise secondary insults have reduced the incidence of hypoxic ischaemic brain damage in fatal childhood brain trauma. In addition, (ii) the amount of hypoxic ischaemic brain damage identified at the post mortem of paediatric brain trauma fatalities correlates proportionately to the total burden of secondary cerebral perfusion pressure insult experience by these patients in the PICU prior to death.

In this chapter, we aim to determine whether the neuropathological features of paediatric brain trauma fatalities differ between cases of different survival duration, and different mode of primary injury mechanisms. In addition, we aim to assess whether the amount of hypoxic ischaemic brain damage identified at post mortem correlated to the total burden of pre-morbid CPP insult in childhood brain trauma.

6.3) METHODS

6.3.1) Original Proposed Methods

All fatal cases already enrolled into the secondary insult study were to be recruited into the neuropathological study. As required by law, all brain trauma fatalities are referred to the Procurator Fiscal in Scotland and the Coroner in England. Permission from the family to perform further detailed research neuropathological investigations

on the samples obtained and retained by the Fiscal or Coroner post-mortem examination would need to be sought before conducting post-mortem examinations. We had planned to ask the Procurator Fiscal or Coroner for access to the post-mortem examination report (including the detailed neuropathological report) after the Fiscal or Coroner post-mortem examination had been carried out.

Laboratory based investigation was to be conducted at the Department of Pathology, Neuropathology Section, Western General Hospital, Edinburgh. A pre-designed proforma was to be used for each case to collect standardised information relating to the detailed pathological features as described previously by Adams and co-workers [369]. Histology was to be examined from the cerebrum, cerebellum, and spinal cord, using both haematoxylin and eosin (H&E) and β -APP immunohistochemistry. In addition, clinical and demographic details, and physiological recordings in minute resolution collected during their intensive care stay prior to death were to be correlated with the recorded pathological findings. Local ethics and hospital management committees approved the study.

6.3.1.1) Problems Encountered During Recruitment

Since the highest anticipated fatality rate of childhood brain trauma is up to 20% [1, 145, 146], the maximum possible number to be recruited for detailed neuropathology investigations from the two centres over a two-and-a-half-year period would still be small. Fatality rate was lower than expected and there were only 5 deaths among the participants of the secondary physiological insult study during the first year of

recruitment. This period also coincided with the height of the public and media attentions on the Alder Hey organ retention scandals. Despite our attentions to research details and forward planning, recruitment into the neuropathological study suffered greatly. Fiscal or Coronial post-mortem examinations were ordered in four of the five fatal cases from our study. All of these families were approached to seek their permissions to use the specimens collected for research by the principal investigator who was also a senior paediatrician. Following verbal explanation of the neuropathology study protocol, written study information sheet was also given to the families. Only two of these four families (50%) gave their consent for the autopsy specimens to be used in research. One family refused consent to a hospital post-mortem following the Fiscals' decision on not ordering a fiscal autopsy. This participation rate is significantly less than the projected figures based on previous data [377] and in contrast to the views of a significant proportion of families involved in the Alder Hey Inquiry where they had indicated their willingness to grant consent for organs that had been kept for research had they been asked [378].

6.3.2) Actual Study Methods

Once the difficulty in prospectively recruiting fatal brain trauma cases with detailed physiological data into the study was realised, the methodology was modified so that most of our hypotheses could be tested using an archival collection of post-mortem information and specimens collected from 50 childhood brain trauma fatalities held within the Neuropathology Section of the Department of Pathology at Western General Hospital over a thirteen-year period between 1990 and 2003. Utilising the

same pre-designed proforma, standardised information relating to the principal pathological features was collected. The previously proposed histological examination was conducted from the cerebrum, cerebellum, and spinal cord, using both haematoxylin and eosin (H&E) and β -APP immunohistochemistry. Non-parametric statistical analyses were conducted because the distribution of data did not follow normal distribution. Mann Whitney U Test was used for continuous variables while Fisher's Exact Test was used for nominal data examined in dichotomies.

I acknowledge that this modified methodology does not allow determination of the relationships between neuropathological findings and pre-morbid physiological insults in fatal childhood brain trauma.

The position on conducting research on archival necropsy materials i.e. organs and tissue retained before December 2000 has been clarified by reports and guidelines published by the Independent Review Group on the Retention of Organs at Post-mortem in Scotland (The McLean Review) [379] and the Medical Research Councils [380]. They acknowledge the difficulties to seek retrospective consents from families for the use of archival tissues for ethically legitimate research or teaching purposes. As a result, they recommended that these material may be used for research provided that ethical approval has been granted by the local ethical committee for the proposed project and the specimens are used in an anonymous and unlinked manner [379, 380]. Following the guidance from these publications, ethical approval for the modified aims and methodology of the study was obtained prior to

commencing data collection and analyses. The study was in accordance with the Human Tissue (Scotland) Act 2006.

6.4) RESULTS

6.4.1) Mode of Brain Trauma and Survival Durations

Mode of brain trauma and survival duration were known in all 50 cases, of which 7 were due to falls, 31 were the result of road traffic accidents, 9 cases were sustained from non-accidental injuries, and 3 were due to other trauma (such as sporting injury). 31 of the 50 cases survived less than 24 hours, of which 19 suffered an instantaneous death.

6.4.2) Distributions of the Different Pathological Findings

49 cases had detailed documentation of all neuropathological features and table 6.1 summarised the distribution of the pathological findings. Brain swelling was the commonest finding as demonstrated in 46 of the 49 cases (94%). The most frequent signs of intracranial hypertension were tentorial and tonsillar hernia. None of the 49 cases demonstrated any infarct without evidence of raised ICP. Supertentorial subarachnoid haemorrhage were the most frequently identified intracranial bleeding site occurring in 73% of cases. 31 of 47 cases had evidence of hypoxic ischaemic brain damage of which 7 were mild, 8 were moderate, and 16 were severe.

Information regarding skull fracture was available in only 34 cases of which half had skull fracture identified.

Table 6.1:

Pathological Features	Numbers (%)
Brain Swelling	46 (94%)
Supertentorial Haematoma	40 (82%)
Evidence of Raised ICP	33 (67%)
Hypoxic Ischaemic Brain Damage (Documented in 47 cases only)	31 (66%)
Brain Contusion	30 (61%)
Infratentorial Haematoma / Burst Lobe	19 (39%)
Intracerebral Haematoma	16 (33%)
Diffuse Axonal Injury	12 (24%)
Supertentorial Burst Lobe	11 (22%)
Gliosis	3 (6%)
Acute Vascular Injury	2 (4%)
Microglial Activation	1 (2%)
Boundary Zone Infarct	1 (2%)

6.4.3) Comparisons of the Neuropathological Features Between Different Survival Durations

6.4.3.1) Survival Less than 24 Hours vs. Survival of 24 Hours or More Post Injury

31 of the 49 cases examined survived less than 24 hours following brain trauma. Table 6.2 detailed the important and statistical significant pathological feature comparisons.

Table 6.2:

	Survival < 24 hours	Survived 24 hrs or more	Significance
Age (months)	90.6	69.6	$p = 0.160$ (Mann Whitney U)
Boys	17 / 31	12 / 18	$p = 0.768$ (Fisher's Exact)
RTA	24 / 31	7 / 18	$p = 0.011$ (Fisher's Exact)
NAHI	3 / 31	6 / 18	$p = 0.054$ (Fisher's Exact)
Diffuse Hypoxic Ischaemic Brain Damage	11 / 30	9 / 17	$p = 0.229$ (Fisher's Exact)
Diffuse Axonal Injury	3 / 31	9 / 18	$p = 0.004$ (Fisher's Exact)
Mean Total Contusion Index	11.23	14.74	$p = 0.680$ (Mann Whitney U)
Evidence of RICP	17 / 31	16 / 18	$p = 0.025$ (Fisher's Exact)
Left Supratentorial Extradural	2 / 31	6 / 18	$p = 0.039$ (Fisher's Exact)
Right Supratentorial Extradural	0 / 31	7 / 18	$p = 0.001$ (Fisher's Exact)
Left Supratentorial Subdural	7 / 31	12 / 31	$p = 0.006$ (Fisher's Exact)
Right Supratentorial Subdural	6 / 31	11 / 18	$p = 0.006$ (Fisher's Exact)
Left Infratentorial Subdural	1 / 31	4 / 18	$p = 0.054$ (Fisher's Exact)
Right Infratentorial Subdural	1 / 31	4 / 18	$p = 0.054$ (Fisher's Exact)

Evidence of raised ICP was found in 55% of cases surviving less than 24 hours post injury but it was evident in 89% of cases surviving more than 24 hours ($p = 0.025$). 16 of the 18 cases (89%) surviving more than 24 hours had evidence of tonsillar herniation bilaterally while it was found in only 29% of cases surviving less than 24 hours ($p < 0.001$). Left sided tentorial hernia was identified more frequently among cases surviving more than 24 hours post injury ($p = 0.04$).

6.4.3.2) Instantaneous Deaths vs. Non-Instantaneous Deaths

19 of the 49 cases examined suffered an instantaneous death. Table 6.3 summarised the important and statistically significant pathological features between the dichotomies.

Table 6.3:

	Instantaneous Deaths	Non-Instantaneous Deaths	Significance
Age (months)	74.5	57.5	$p = 0.159$ (Mann Whitney U)
Boys	12 / 19	17 / 31	$p = 0.769$ (Fisher's Exact)
RTA	16 / 19	15 / 30	$p = 0.012$ (Fisher's Exact)
NAHI	0 / 19	9 / 31	$p = 0.008$ (Fisher's Exact)
Diffuse Hypoxic Ischaemic Brain Damage	4 / 18	16 / 28	$p = 0.032$ (Fisher's Exact)
Diffuse Axonal Injury	0 / 19	9 / 30	$p = 0.014$ (Fisher's Exact)
Mean Total Contusion Index	12.58	12.55	$p = 0.834$ (Mann Whitney U)
Evidence of RICP	10 / 19	23 / 30	$p = 0.119$ (Fisher's Exact)
Right Supratentorial Extradural	0 / 19	7 / 30	$p = 0.016$ (Fisher's Exact)
Left Supratentorial Subdural	3 / 18	16 / 30	$p = 0.016$ (Fisher's Exact)
Right Supratentorial Subdural	2 / 18	15 / 30	$p = 0.011$ (Fisher's Exact)

Fewer cases of instantaneous deaths had diffuse hypoxic ischaemic brain damage than those among non-instantaneous deaths (Table 6.3). Significantly more instantaneous deaths (72%) had either no evidence or only mild degree of hypoxic ischaemic brain damage when compared with cases from non-instantaneous deaths (34%) ($p = 0.02$, Fisher's Exact Test). Diffuse traumatic axonal injury was not found in any of the instantaneous deaths but was identified in 67% of non-instantaneous deaths ($p < 0.01$, Fisher's Exact Test).

Evidence of raised ICP did not differ significantly between the two dichotomies (Table 6.3). Significantly fewer instantaneous deaths demonstrated evidence of tentorial and tonsillar hernia when compared with non-instantaneous deaths (22% vs. 63% for left sided tentorial hernia, $p < 0.01$; 26% vs. 63% for right sided tentorial hernia, $p = 0.02$; 22% vs. 70% for tonsillar hernia bilaterally, $p < 0.01$).

6.4.4) Comparisons of the Neuropathological Features Between Different Modes of Brain Injury

6.4.4.1) Road Traffic Accidents (RTA) vs. Non-RTA

31 cases sustained their brain trauma from road traffic accidents (RTA). RTA fatalities were significantly older than those from non-RTA (median age 105.2 months vs. 32.9 months respectively, $p < 0.01$, Mann Whitney U Test).

Significantly more instantaneous deaths were the result of RTA (52%) when compared with non-RTA (13%) ($p < 0.01$, Fisher's Exact Test). Similarly, there were significantly more RTA fatalities who survived less than 24 hours post injury than non-RTA fatalities (77% vs. 38%, $p < 0.01$).

There was also a trend to suggest that RTA fatalities tend to have more moderate or severe contusion than the non-RTA deaths (32% found in RTA fatalities vs. 7 % in non-RTA fatalities, $p = 0.07$). The mean total contusion index was 13.2 and 12.8 in RTA and non-RTA deaths respectively and did not show any statistical significance ($p = 0.36$). Brain trauma deaths from RTA and non-RTA had similar evidence of raised ICP. No significant difference was found in the incidence of haematomata and burst lobes of all intracranial locations between RTA and non-RTA fatalities.

6.4.4.2) Accidental Head Injuries vs. Non-Accidental Head Injuries (NAHI)

9 cases sustained their brain trauma from non-accidental head injuries (NAHI). They were significantly younger than those cases with brain trauma from accidental causes (median age of 3.6 weeks in NAHI cases compared to 107.2 weeks in accidental head injuries, $p < 0.001$, Mann Whitney U Test).

None of the NAHI suffered an instantaneous death while 46% of the accidental head injuries suffered an instantaneous death ($p < 0.01$, Fisher's Exact Test). There was a

trend to suggest more accidental head injuries cases surviving less than 24 hours than the NAHI cases (68% in accidental head injuries vs. 33% in NAHI, $p = 0.06$).

Contusion was found significantly more frequently among accidental brain trauma fatalities than NAHI (71% in accidental brain trauma fatalities vs. 13% in NAHI, $p < 0.01$, Fisher's Exact Test). No significant difference was found in the evidence of raised ICP between the two causes of brain trauma fatalities.

Apart from the highest frequency of left sided supratentorial subdural haematomata found among NAHI fatalities (75% in NAHI vs. 33% in accidental head injury, $p = 0.04$, Fisher's Exact Test), no significant difference was found in the incidence of haematomata, and burst lobes of other intracranial locations within the dichotomy.

6.4.4.3) Falls vs. Non-Falls

Fall was the cause of brain trauma in 7 cases. The median age, sex distribution, and survival duration did not differ between fatalities due to falls and non-falls.

Death from falls and non-falls had similar incidence of signs of raised ICP. Significantly more fatalities from falls had left sided supratentorial extradural haematomata when compared with brain trauma death caused by non-fall mechanism (57% in falls vs. 10% in non-falls, $p < 0.01$ Fisher's Exact Test). Right sided supratentorial subarachnoid haematomata were found among all falls related fatalities but demonstrated in only 56% of non-fall related death ($p = 0.04$, Fisher's

Exact Test). No significant difference was found in the incidence of haematomata and burst lobe in other intracranial locations between the 2 groups.

6.5) DISCUSSIONS

Detail neuropathological examination of fatal childhood brain trauma provides an ideal opportunity to understand how different injuring mechanisms, survival durations, brain maturity, and clinical management affect the distribution of neuropathological patterns. To the best of my knowledge, there is no other systematic neuropathological report of fatal paediatric traumatic brain injury which included accidental injuries since Graham's report [60] in the literature until our current study. Our current series differ from Graham's cohort [60] in several ways. Our series collected necropsy data from fatal childhood brain trauma which included younger infants and those who died of non-accidental brain trauma. In addition, our cohort had shorter survival duration (62% of the current series survived less than 24 hours while the median survival period was 48 hours in Graham's cohort [60]), originated from a different geographic area (Eastern vs. Western Scotland), and the fatalities had occurred at least 10 years later than those cases from Graham's group [60]. Despite these differences, important information on both primary brain injury and secondary brain insults can be obtained from data comparisons between these studies. Furthermore, the composition of our current series allowed important comparison to be made between cases of different survival durations and mode of brain trauma and allowed the difference in the neuropathological pattern among these groups to be highlighted.

6.5.1) Diffuse Brain Swelling

Diffuse brain swelling is the commonest pathological finding of fatal brain trauma at post mortem and it is identified more frequently among childhood brain trauma than adult TBI. Graham and co-workers previously reported finding brain swelling among 70% of the cases of fatal paediatric brain trauma [60] which was 4 times more frequent than the figure reported from their main adult brain trauma fatality cohort [369]. Brain swelling was found in 94% of our current cohort which was even higher than that found in Graham's series [60] which excluded patients who did not survive long enough to reach neuro-surgical care. This finding confirmed that the immature brains of infants and children were prone to developing brain swelling following trauma, and brain swelling may occur any time after trauma regardless of the survival period.

Different duration of survival and mode of brain trauma did not have any differential effect on the prevalence of brain swelling among our current series. This was not an unexpected finding given that cerebral oedema is a generic clinical manifestation of various pathological processes initiated by brain trauma.

6.5.2) Hypoxic Ischaemic Damage

Ischaemic brain damage is a common post mortem finding of adults and children who died from brain trauma. Despite advancement in resuscitation and treatment of brain trauma, Graham and colleagues reported that ischaemic brain damage identified at post mortem was as common in the early 1980s as it was in the late 1960s and early 1970s in the West of Scotland [58]. The prevalence of ischaemic brain damage in our current series (66%) is lower than that previously reported by Graham's paediatric group (80%) [60] but majority of the ischaemic brain damage identified in both series were of moderate to severe severity (51% in our series vs. 61% in Graham's group [60]).

The difference in prevalence of ischaemic brain damage between our current series and that of Graham's paediatric group may be a mere reflection of the improvement of resuscitation measures and brain trauma treatment with time. However, the similarity in the prevalence of moderate to severe ischaemic brain damage identified in both series would suggest that the changes in resuscitation training and provision, and neuro-intensive care treatment of brain trauma patients over the past two decades had failed to prevent ischaemic brain damage of this severity.

The difference in survival durations between our current series and that of Graham's cohort [60] may better explain the difference observed in the overall ischaemic brain damage prevalence. The survival time composition of our cohort enabled us to confirm that hypoxic ischaemic brain damage requires time to evolve. In our current

cohort, we found that although hypoxic ischaemic brain damage was identifiable in cases of instantaneous death, significantly fewer cases of instantaneous death had evidence of diffuse hypoxic ischaemic brain damage when compared to those of non-instantaneous deaths ($p = 0.03$). Instantaneous death from fatal brain trauma represented the most severe brain trauma when the primary brain injury was too severe to survive and these cases did not have sufficient time for secondary brain insult to evolve. Secondary brain insult should, therefore, contribute little towards the pathological findings in this group. Graham's cohort did not include any instantaneous deaths [60]. All their cases had, therefore, survived long enough to allow ischaemic brain damage to evolve and be identified at post mortem which may explain the higher prevalence of ischaemic brain damage described in Graham's report.

We also demonstrated that survival beyond 24 hours did not increase the ischaemic brain damage prevalence when compared with those surviving less than 24 hours ($p = 0.23$). Patients surviving less than 24 hours represented a group of patients whose primary brain injury was not severe enough to cause death instantaneously, and although their survival time was short, it was long enough for secondary hypoxic ischaemic brain damage to develop. It also reflected that any patients who survived the initial brain trauma were as susceptible to ischaemic brain damage as those who survived longer than 24 hours and highlighted the importance of diligent acute trauma care (including pre-hospital care) to normalise the systemic and cerebral homeostasis early following brain trauma.

We were unable to correlate the amount of ischaemic damage present at post mortem with the patients' pre-morbid CPP insult because they were archival cases and their pre-morbid physiological insult data were not available.

6.5.3) Diffuse Traumatic Axonal Injury

Diffuse traumatic axonal injury is defined as widespread damage to axons in the white matter of the brain and is a well recognised sequela of traumatic brain injury [381-385]. The first clinical description of primary shearing injury of the axons was reported by Strich from a series of demented patients following severe brain trauma in the 1950s [383], but her concept was actually hypothesized earlier in the 1940s by a physicist called Holbourn who predicted the brain's peculiar vulnerability to shearing injuries given the extremely low modulus of neural tissue to shearing deformation and a very high modulus of compressibility [386, 387]. Experimental evidence over the years supported Holbourn's theory of 'swirling' motion of the brain within the skull during impact. Furthermore, evidence indicated that the shearing injury to the brainstem would only occur in more severe injuries than those required to initiate shear strains within the cerebral white matter [388]. Thus, primary brainstem injury usually occurs in concomitant with shearing injuries of the cerebrum except in rare circumstances where a rapidly fatal tear occurs in the pontomedullary junction [367].

The prevalence of diffuse traumatic axonal injury in our study (24%) was similar to that of Graham's paediatric report (22%) from Western Scotland [60] but was almost

double the prevalence (13%) reported in Scottish adult brain trauma fatality [369]. This difference in diffuse traumatic axonal injury prevalence between paediatric and adult brain trauma fatalities may be explained by the progressive increase in myelination of the immature nervous system through development. The increase in brain weight over the first 4 years of life is mainly due to an increase in white matter myelin. Grey matter is firm and cellular whilst poorly myelinated white matter is more gelatinous and of slightly different density and so with acceleration-deceleration injury they swirl at different velocities giving rise to tears in the parenchyma between the grey and white matter [389]. With this tendency of the infants axons to stretch more than the fully myelinated adult fibres [390], it is easy to understand why white matter injuries are also observed more frequently in infants sustaining non-accidental brain injury as demonstrated by neuroimaging.

White matter shearing injuries are particularly seen in corpus callosum, superior cerebellar peduncle, and in the midbrain. Shearing injury of the midbrain results in loss of consciousness from the moment of impact because sudden rotation of the cerebral hemispheres upon the more anchored midbrain causes a primary stem injury ensuring the occurrence of sudden coma [388]. The clinical picture consists of loss of consciousness, dilated pupils, decorticate posture, and abnormalities of blood pressure, pulse and respiration.

When comparing the prevalence of diffuse traumatic axonal injury among cases of different survival durations, we found that none of the instantaneous deaths had evidence of diffuse traumatic axonal injury but it was more frequently identified in

deaths with a survival period longer than 24 hours. This was consistent with the findings of an earlier Scottish study where only 4% of diffuse traumatic axonal injury was found in patients surviving less than 24 hours after brain trauma [391]. Similar preponderance of longer survival duration in cases with diffuse traumatic axonal injury was also described in a later Australian study where 82% of the brain trauma cases with evidence of diffuse axonal injury survived more than 24 hours [392]. Thus, diffuse traumatic axonal injury requires time to evolve and it is evident that diffuse traumatic axonal injury has several different microscopic appearances dependent upon the survival interval [93]. Axonal retraction balls are typically found in fatal brain trauma of short survival durations (in terms of days) and are formed by exuded axoplasm from shearing of axons by traction forces. They may be detected among brain trauma fatalities who had survived for as short as 35 minutes [374]. The appearance of diffuse traumatic axonal injury of intermediate survival duration of several days to weeks post brain trauma is characterised by microglial scars while fibre tracts degeneration in the white matter is only evident in patients who had survived for many weeks to months following brain trauma.

The relationship between early feature of diffuse traumatic axonal injury (axonal swelling) and survival time after brain trauma has been previously investigated. Wilkinson and colleagues demonstrated that axonal swelling size was directly proportionate to survival time until 85 hours post injury when the size of axonal swelling plateaued in a cohort of 66 fatal brain trauma [393]. This positive correlation between axonal swelling size and survival time was also confirmed by another British group who examined brain sections from 63 (adult and childhood)

brain trauma fatalities [394], and Gorries' group from Sydney who investigated the characteristics of axonal injury among 32 children dying from motor-vehicle related brain trauma [395]. Developmental difference in myelination between the immature and adult brains did not appear to influence the relationship between axonal size and survival time because Gorries' group also compared axonal sizes from various survival times between paediatric and adult brain trauma fatalities and found no difference in the sizes of axonal swelling between the two groups [395].

Majority (67%) of our cases with evidence of diffuse traumatic axonal injury sustained their injury from road traffic accidents while falls infrequently resulted in diffuse axonal injury. This finding was consistent with figures described by previous reports [385, 391, 392]. Denny-Brown and Russell reported that in experimental brain trauma, greater force was required to initiate shearing injury in the fixed head than when the head was free to move [396]. Angular acceleration of the head is, therefore, more likely to occur during road traffic accidents with the potentials for whiplash and similar injuries than during a fall when the head is relatively stationary at the moment of impact.

Genarelli's group confirmed the importance of angular acceleration of the head in the production of diffuse axonal injury [397, 398]. They utilised angular acceleration of the head in subhuman primate and reproduced diffuse axonal injury that was pathologically identical to those observed in human brain trauma [397, 398]. Duration of angular acceleration differs significantly between RTA and falls, and it is thought to be important to the production of diffuse traumatic axonal injury.

Genarelli's earlier experiments only reproduced contusions in the frontal and temporal lobes as well as the occasional intracerebral haematoma from using short acceleration pulse. However, with increasing the acceleration, they were able to reproduce torn bridging veins and the formation of rapidly fatal subdural haematoma while increasing the duration of the applied acceleration pulse resulted in the reproduction of prolonged unconsciousness and diffuse axonal injury [398]. They also found that subdural haematoma occurred less frequently with increasing angular acceleration durations [398] which was consistent with the clinical observations made by Adams' group where significantly lower incidence of intracranial haematoma was found among patients with diffuse traumatic axonal injury [391].

The precise mechanisms responsible for the production of diffuse traumatic axonal injury in brain trauma have been the subject of intense investigations since its first clinical description in the 1950s. Although the time course of pathological changes in diffuse traumatic axonal injury has been defined by Adams [381] and many clinico-pathological studies supported the concept of primary mechanical shearing injury to the axon [93, 381, 391], many other authors argued that primary axotomy only represented one possible mechanism and suggested that secondary insults such as hypoxia, cerebral oedema, vascular disruption from raised intracranial pressure and subsequent tentorial herniation, and other metabolic disturbances like hypoglycaemia and seizures were causes of delayed secondary axotomy [376, 399]. Adams and colleagues evaluated 45 cases of non-missile head injury in the early 1980s but found no difference in the incidence of ischaemia or cerebral oedema between cases with or without diffuse traumatic axonal injuries [391]. In addition,

they found significantly fewer cases with diffuse traumatic axonal injury had evidence of intracranial hypertension than those without diffuse axonal injury [391].

The concept of secondary axotomy has regained research interests over the past decade when Kaur and Oehmichen independently described hypoxia induced axonal injury without brain trauma [375, 400], and Dolinak and co-workers demonstrated axonal injury among 13 fatal non-traumatic coma attributed to hypoglycaemia [376]. Although both Kaur and Oehmichen's series described hypoxia induced axonal injury without brain trauma [375, 400], they did not explore the contributory effects of confounding factors such as cerebral oedema, raised ICP, and internal herniation [401, 402]. Dolinak and colleagues addressed these issues in their investigations of the brains from 44 fatalities due to cardiac arrests, status epilepticus, carbon monoxide poisoning, and controls cases who died from extracranial causes [376]. They found that patients with evidence of axonal injury also had evidence of raised intracranial pressure suggesting that hypoxia per se was an unusual cause of axonal injury [376]. These studies have highlighted that not all post traumatic axonal injury is caused by the primary mechanical trauma but secondary insult also contributes to the evolution of diffuse axonal injury. Thus, the analysis of fatal brain trauma must include a full assessment of both primary injury and secondary insults which requires the full clinico-pathological details including injuring mechanisms, survival durations, physiological insult measurement, and detailed neuropathological studies.

6.5.4) Limitations

Data used in our study originated from an archival collection of post-mortem reports and specimens obtained from fatal childhood brain trauma over a thirteen-year period. A number of different pathologists would have been involved in the post-mortem of these cases, and although standardised post-mortem examinations were conducted, invariably some findings were not recorded in the post-mortem report resulting in missing data and potentially introducing some bias into the data analyses. From our data set, one case had incomplete documentation of pathological features and was excluded from the analyses. Evidence of skull fracture was not documented in 32% of cases, as a result, we were unable to investigate the relationship between skull fracture and other pathological features of childhood brain trauma, and to compare its prevalence to previous reports in the literature. This has not affected testing of our hypotheses but skull fracture is an important pathological feature of impact injury and its presence is often associated with other significant intracranial injury such as intracranial haematomata. Thus, the knowledge of its prevalence and correlation with other brain damage features are still important in any detailed neuropathological study of brain trauma fatalities.

Another limitation is the small cohort size. Our cohort consisted of 50 cases of paediatric brain trauma with different modes of injuring mechanisms, age, and survival duration. Once the cohort was subdivided into dichotomies, each sample size became fairly small making it difficult to obtain statistically meaningful findings for certain comparisons. We have however, been able to demonstrate a number of

important and statistically significant differences in the observed pathological features between different survival durations. This was particularly useful given majority of the reports in the literature were from patients surviving long enough to reach neurosurgical centres and excluded instantaneous deaths.

6.6) CONCLUSIONS

Very few detailed reports of neuropathological features of fatal childhood brain trauma exist in the literature even though brain trauma remains the commonest cause of paediatric deaths. Brain swelling, moderate to severe hypoxic ischaemic brain damage, and diffuse traumatic axonal injury continue to be common findings in fatal childhood brain trauma and they all occur as frequently in the 1990 and early 2000s as they were in the 1960s and early 1980s despite significant changes to the clinical management of these patients over the past 2 decades including advances in resuscitation and trauma care with an increased emphasis on minimising secondary insults. Children dying from brain trauma are more susceptible to brain swelling than adults, but it is unclear whether there is any age related difference in the susceptibility to the development of brain swelling throughout childhood.

We confirmed that hypoxic ischaemic brain damage and diffuse traumatic axonal injury required time to evolve and rarely found among instantaneous deaths. Survival period beyond 24 hours did not increase the prevalence of hypoxic ischaemic brain damage indicated that brain injured children who survived the initial brain trauma would be susceptible to ischaemic brain damage and highlighted the

importance of diligent acute trauma care (including pre-hospital care) to normalise the systemic and cerebral homeostasis following brain trauma. The prevalence of diffuse traumatic axonal injury was highest among deaths with survival period beyond 24 hours following the initial injury but it remains unclear whether secondary axonal injury contributed towards this finding.

Further study is required to determine the relationships between secondary CPP insults and hypoxic ischaemic brain damage in fatal childhood brain trauma, and how maturational difference in the nervous system throughout childhood influences the susceptibility of developing different neuropathological features such as brain swelling, hypoxic ischaemic brain damage and diffuse traumatic axonal injury after brain trauma.

CHAPTER 7: MODULATING EFFECTS OF PHYSIOLOGICAL, GENETIC, AND BIOCHEMICAL FACTORS ON THE SEQUELAE OF CHILDHOOD BRAIN TRAUMA

7.1) INTRODUCTION

Brain trauma occurs frequently, and remains the commonest cause of death and disability in children over 1 years of age [46, 177]. Many survivors of paediatric brain trauma suffer from significant physical, emotional, and psychological morbidities [52-55]. Although injury prevention has dramatically reduced the number of trauma deaths, accidents will continue to occur. It is, therefore, essential to optimise brain trauma management and to develop new therapies so that when brain trauma occurs, the potentially devastating outcome may be improved.

Basic principles of neuro-intensive care for both adults and children remain unchanged since its introduction in the late 1970s [76] with an emphasis on minimisation and prevention of secondary ischaemic insults [74, 75]. Search for novel neuro-protective strategies have been extensive but disappointing mostly because of the difficulty to translate animal research and in-vitro study results into clinically relevant therapies. One possible explanation for the bench-to-bedside translational difficulty is that these promising laboratory neuro-protective strategies mostly target grey matter damage without affecting white matter injuries which may be more important to neurological recovery after brain trauma. Furthermore, most of these potential therapies have targeted a single pathway among the complex cascade

of brain trauma induced pathophysiological responses. Reduction in one injuring pathway may only exacerbate other brain injuring mechanisms which will not improve the overall recovery.

This thesis utilizes clinical studies to investigate multiple outcome determinants of childhood brain trauma. This chapter aims to address how physiological, genetic, and biochemical factors modulate the sequelae of childhood brain trauma.

7.2) CEREBRAL ISCHAEMIA AND BRAIN TRAUMA RECOVERY

Prevention and minimisation of cerebral ischaemia are the treatment goals of modern neuro-intensive care [74, 75]. However, cerebral ischaemia is difficult to quantify at the bedside. Clinicians, therefore, use cerebral perfusion pressure (CPP) derangement as an indirect measure of cerebral ischaemia and the critical care management of brain trauma patients emphasizes the importance to maintain an adequate CPP. Treatment thresholds for CPP and ICP have been proposed and become widely accepted in adult practice [74] but they have not been validated. This is possibly because of the previous lack of suitable quantification methods to measure the total burden of CPP or ICP insult.

Methodology to quantify deranged CPP has previously been limited to assessing one dimension of the insult (for example, duration or magnitude) at any one time. Despite the limited assessment methods available, the relationships between CPP derangement and brain trauma outcome in adults and children have been well

described [63, 90-92, 207]. Some researchers, therefore, questioned the need to develop more robust quantification methods that enable assessment of the total burden of CPP insult i.e. measuring both the insult duration and magnitude spontaneously.

The importance to quantify the total burden of CPP insult after brain trauma was apparent when we assessed brain trauma children's APO E genotypes and their relationships with CPP insult and outcome [261]. The duration or frequency of CPP derangement (i.e. one dimensional insult assessment) did not differ between children carrying the different APO E alleles. However, when the total burden (i.e. duration and magnitude) of CPP insult was quantified using the novel cumulative pressure time index (PTI), the APO E genetic polymorphisms were clearly associated with different burden of CPP insult with the e4 carriers having the least amount while the e3 homozygotic children experiencing the largest insult burden [261].

Our novel PTI is the first described method to measure the total burden of CPP insult by taking into account both the duration and magnitude of the insult [203, 204]. It is easy to calculate and allows comparison of insult between different age groups [204], which is particularly important in paediatric brain trauma research. With the PTI, we confirmed the importance of cerebral ischaemia on brain trauma recovery. Brain trauma children with unfavourable outcome had suffered significantly more CPP insult than those who had good recovery [204]. This was true regardless of the patients' APO E genotypes [261]. Furthermore, PTI has enabled us to define age-related critical CPP thresholds [204].

Unlike adult practice, there are no accepted CPP treatment thresholds for infants and children because of the lack of information on the normal age-related intracranial haemodynamics. The desired CPP treatment thresholds in childhood brain trauma are often extrapolated from the non-validated adult threshold or have been age-adjusted anecdotally by individual clinicians.

Our proposed age-related theoretical minimum levels for CPP were based upon normal physiology where ICP should have minimal contribution to the CPP, which must, therefore, equate to the minimum age-related mean arterial blood pressure [92, 192-195]. In brain trauma, if the cerebral autoregulation remains intact, the body should, theoretically, be able to regulate the mean arterial blood pressure to compensate for any rises in the ICP and to ensure this minimum CPP level is maintained. Brain trauma critical care management aims to ensure an adequate CPP is maintained in addition to treating intracranial hypertension [74, 75]. In patients with impaired cerebral autoregulation, intensivists use vasopressors to artificially drive mean arterial blood pressure to super-physiological levels to maintain the target CPP [74, 75].

Using the PTI, we demonstrated that insult measured using these theoretical age-related CPP thresholds correlated best with unfavourable outcome and altering these thresholds did not improve the outcome prediction performance of the calculated insult [204]. CPP levels lower than these critical thresholds will, therefore, cause ischemic insult thereby affecting recovery. This proved that our theoretical age-

related thresholds were the minimum CPP levels by age where cerebral perfusion may be achieved [204]. CPP treatment thresholds in the paediatric population must, therefore, be higher than these critical thresholds to avoid cerebral ischaemia, but further studies are required to define the best age-related therapeutic CPP thresholds.

Age-related CPP insult was found to occur very frequently in our cohort indicating that existing brain trauma management was ineffective in its prevention. The urgent need to define CPP treatment thresholds was also confirmed by our post-mortem study. We found that despite diligent efforts to improve paediatric trauma resuscitation and neuro-intensive care provision in Scotland, hypoxic ischaemic brain damage continued to be a frequent finding in paediatric brain trauma fatalities with little change in the prevalence over the past 20 years [60]. Since none of the potential novel neuro-protective strategies are likely to be translated successfully into clinically effective therapies overnight, the best chance to improve brain trauma outcome in the foreseeable future relies upon optimisation of the CPP treatment thresholds to prevent cerebral ischaemia. The development of PTI will not only benefit paediatric brain trauma research but also enable validation of the well-accepted adult CPP treatment threshold and to assist development of a threshold that best prevent cerebral ischaemia which may then improve outcome.

7.3) Potential Modulating Mechanisms of APO E Genetic Polymorphisms on Brain Trauma Outcome

The human genome project suggests that genetic factors do not only predispose individuals to certain pathological conditions such as various cancers or emphyzema but also govern the body's response to injuries or disease processes and medical treatments [403]. Understanding how genetic polymorphisms affect the host's response to brain trauma and its treatment may enable potential novel treatment points to be identified. Most previous clinical studies of the APO E genetic polymorphisms had concentrated on assessing the association between poorer outcome and the possession of the APO E e4 allele [141-143]. Majority of the potential mechanisms of action have only been assessed using animal models and in-vitro cell cultures. Only a few clinical and post-mortem studies have attempted to investigate potential mechanisms of action.

7.3.1) Potential Mechanisms Assessed by Clinical or Post Mortem Studies in the Literature

7.3.1.1) Apolipoprotein E Polymorphisms and Coagulation

Apolipoprotein E genetic polymorphisms have varying influence on blood coagulation. Vitamin K concentrations vary between dialysis dependent renal failure patients possessing the different APO E alleles with the highest concentration found among the APO E e2 carriers, intermediate amount found in the e3 carriers, and the

least amount found in the e4 carriers [404]. The APO E genotypes have also been reported to affect the prothrombin times in patients with alcoholic cirrhosis [405]. Significant prolongation of both the international normalised ratio (INR) and the ratio of the partial thromboplastin times were found among APO E e4 carriers of stroke patients when compared to patients without this allele in a recent retrospective study involving 578 adult stroke sufferers [406].

Since coagulopathy post brain trauma is an independent predictor of vascular complication and poor outcome [407-409], the influence of APO E genetic polymorphisms on neurological recovery after brain injury may potentially be mediated through allelic specific effects on coagulation. Liaquat and co-workers assessed the effects of APO E genotypes on the size of acute intracranial haematomas among 129 brain trauma patients and reported that larger sized haematomas (3.1 cm vs. 2.5 cm, $p = 0.0039$) were found among those in possession of one or more APO E e4 allele [410]. Coagulation status will influence the size of intracranial haematomas after brain trauma, but the mechanisms of primary injury will also determine the size of the haematomas in these patients. It may be possible that the mechanisms of injury were more severe among those possessing APO E e4 allele resulting in larger haematomas in these patients.

Traumatic coagulopathy has been proven to be a form of disseminated intravascular coagulation (DIC) triggered by the release of tissue thromboplastin by injured brain tissue [411, 412]. Tissue thromboplastin may also stimulate intravascular microthrombosis which has also been confirmed recently to be an important

secondary insult following non-fatal brain trauma and appears to correlate with post-traumatic ischaemic neuronal death [413-415]. Thus, traumatic coagulopathy is not limited to regions of initial mechanical injury in brain trauma but may induce a series of secondary insult distant to the original injury site. Despite previous reports of the relationship between APO E genetic polymorphisms and coagulation, only recently the influence of the APO E genotype on the intravascular coagulation, and in particular intravascular microthrombosis (IMT) following brain trauma have been investigated.

Stein and colleagues conducted detailed neuropathological investigations on 19 post-mortem materials from fatal brain trauma and 18 surgical specimens obtained from contused brain removed during surgical decompression for closed traumatic brain injury [416]. They found that e4 carriers were more likely to have a lower IMT density. Despite having slightly longer prothrombin times and higher coagulation indices, no association was found between this particular allele and the degree of coagulopathy [416]. Stein and colleagues believed that these findings had disproved their postulation of a predisposition to developing DIC and IMT after brain trauma in patients possessing the APO E e4 allele [416]. However, the sample size of their study was small and the sampling sites of both the surgical and autopsy groups were heterogenous limiting the validity of the findings. Further study employing larger sample size and unified sampling site is required to define the true relationship between APO E genotypes and the development of DIC following brain trauma.

7.3.1.2) APO E Genotypes and Post-Traumatic Cerebral Swelling

APO E genotypes have been suggested to affect the degree of post-traumatic cerebral swelling differently because APO E e4 carriers have been found to have greater degree of cerebral swelling related to focal traumatic contusions than the non-e4 carriers [410]. The potential influence of apolipoprotein E on cerebral oedema development has also been demonstrated in animal experiments where APO E knockout mice develop more marked cerebral oedema after experimental trauma than wild-type mice [417]. Diffuse brain swelling was found more frequently in fatal paediatric brain trauma than in adult TBI [60]. A recent paediatric post-mortem study was, therefore, conducted to assess the effect of APO E genotypes on unilateral or bilateral hemispheric swelling in fatal paediatric head injury [418]. No association was found between the APO E e4 allele and post-traumatic brain swelling in this archival post-mortem study of 106 cases [418]. The sample size of this study should have been sufficient to detect a difference of 30% in the proportion with swelling with 80% power, but the actual difference observed was 1% [418]. In a study of 102 adults suffering from intracerebral haemorrhage, McCarron and colleagues demonstrated that although higher mortality rate was found among the APO E e4 carriers than the non-e4 carriers, and larger volume of cerebral oedema was found among the non-survivors, the size of cerebral oedema was not related to the e4 allele [419]. APO E genotypes are, therefore, unlikely to have any genuine influence on the development of cerebral oedema post brain injury.

7.3.1.3) APO E Genotypes and the Conversion of β amyloid Precursor Protein to β Amyloid

The influence of APO E genotypes on the conversion of the neuroprotective β amyloid precursor protein to β amyloid has considerable evidential support over the last decade and has been tested in several post-mortem studies. β APP is expressed constitutively in neurons. Its up-regulation following neuronal injury is observed in perikarya and axons [256]. In certain circumstances and predisposed individuals, β APP may give rise to deposits of β amyloid (β A4), which is known to be toxic to neurons [256]. β A4 deposition have been found in up-to one third of patients who die from brain trauma and a higher frequency of APO E e4 allele is found among those who has β A4 deposition after head injury [420]. The density of β A4 plaques found in fatal head injured patients was associated with the APO E e4 allele in a dose-dependent manner [421]. In a further study comparing β A4 deposition in long-term survivors of head injury with that found in a group of age and APO E genotype matched controls, MacFarlane and colleagues found that β A4 deposits were not more common among those who had survived a previous TBI although β A4 deposits were more common among those possessing APO E e4 allele [422].

7.3.1.4) Limitation of Post-mortem Studies for Assessing Potential Mechanisms of Action

Post-mortem studies limit the investigations of the postulate because clearly, the patient cohort has been selected by death and a bias towards the worst outcome has been introduced.

7.3.2) Potential Mechanisms Assessed by Animal or In-Vitro Studies

Most postulated mechanisms of the APO E genetic influence on neurological outcome after CNS insults have been related to the functions of its gene product, the apolipoprotein E (apoE), which is a major lipoprotein in the CNS [144]. It is involved in the CNS lipid transportation and metabolism [144]. Because the presence of apoE within the cytoplasm of neurons [423], it has also been postulated to interact and regulate the integrity of cytoskeletal proteins [424-426].

In vitro studies have demonstrated that the E3 isoform of apoE binds the microtubule-associated proteins (MAPs) tau, and MAP-2c to form apoE/MAP complex [425, 426]. The region of tau where apoE3 binds to is thought to be responsible for causing self-assembly into the paired helical filament, which is the basic structural component of the neurofibrillary tangles [427, 428]. The E4 isoform on the other hand does not bind with MAPs tau [425]. Apolipoprotein E isoform specific interaction with MAPs tau may, therefore, regulate the rate of formation of

the paired helical filaments and neurofibrillary tangles which are important in the maintenance of the cytoskeletal integrity.

Apolipoprotein E may also protect against oxidative and excitotoxicity injury because experiments involving apoE deficient mice indicate that the lack of apoE increases oxidative injury [429, 430]. Plasma lipoproteins from these mice were more susceptible to in-vitro oxidation than those of wild-type mice, and they have an increased expression of autoantibodies against oxidized lipids when compared to control animals [429, 430]. Furthermore, different isoforms of apoE offered a neuronal cell line different protection against hydrogen peroxide toxicity with the E2 isoform offering the highest degree of protection, while the least protection was observed with the E4 isoform [431]. As a result, APO E genetic polymorphisms have been postulated to modulate brain injury recovery by offering isoform-specific protection against oxidative injury.

In vitro neurite extension of a central nervous system-derived neuronal cell line was increased with apoE3 but not apoE4 [432]. In addition to this potential direct neurotrophic effects on injured neurons, apoE has been shown to enhance the neurotrophic effects of growth factors such as ciliary neurotrophic factor [433].

Laminin is an extracellular matrix protein that affects neuronal adhesion, spreading, differentiation, and growth by binding integrin-type cell surface receptors. It has been shown to interact with apoE in vitro. Furthermore, the laminin-apoE substrate produced higher number of live, attached hippocampal neurons in cell culture than

laminin alone. This suggests that apoE may be important in the development and maintenance of neurons in the CNS by regulating interactions between the neuron and the extracellular matrix [434].

Apolipoprotein E may modulate neuro-inflammation. Its in-vitro immunomodulatory effect suppressed lymphocyte proliferation and immunoglobulin synthesis after mitogenic stimulation [435, 436]. It has also been shown to suppress glial secretion of inflammatory cytokines in an isoform specific and dose-dependent fashion [437]. Recent transgenic animal studies suggested that an enhanced neuro-inflammation was found in the APOE4 transgenic animals when compared to the E3 transgenics [265, 266]. APO E genetic polymorphisms may, therefore, modulate brain injury recovery through different allelic influence on neuro-inflammation.

All these postulates may have important modulating effects on brain trauma outcome but none have been assessed in the clinical setting. Their clinical relevance is, therefore, unclear.

7.3.3) APO E Genetic Polymorphisms And Cerebral Ischaemic Tolerance: Evidence from Bench and Bedside Studies

No previous studies have assessed whether carriers of the different APO E alleles would have different burden of CPP insult after brain trauma. Given the significance of CPP insult on brain trauma outcome and the growing evidence to suggest an APO E e4 allelic association with poorer outcome, we postulated that the e4 carriers

recovered less favourable after brain trauma because the e4 allele affects the host's response to brain trauma and its management causing more CPP insult than patients without this allele. This postulate was disproved when we demonstrated that brain trauma children possessing the e4 allele had experienced the least amount of CPP insult, which were 13.3 times less than the non-e4 carriers [261]. We also observed that the e3 homozygotic children with good outcome did so despite having had nearly 26 times more CPP insult than those who were non-e3 homozygous [261]. In addition, the CPP insult level experienced by the APO E e4 carriers with unfavourable outcome was 22 times less than the non-e4 carriers and it was at a level that should have conferred good recovery [261].

The observed relationships between APO E genotypes, CPP insult and outcome suggests that APO E genetic polymorphisms may affect the host's cerebral ischemic tolerance differently and the prevention of cerebral ischaemia may be even more important in the APO E e4 carriers after brain trauma. This postulate supports a recent observation from transgenic animal research which showed an increased risk of brain damage from ischemic injury in the APO E e4 transgenic mice when compared to the e3 transgenic animals [248].

7.4) BRAIN TRAUMA BIOMARKERS AND OUTCOME

Because the complex cascade of biochemical and molecular pathophysiological processes initiated by brain trauma are regulated and modulated through biomediators, many intensivists hope to utilize these biomarkers for objective

quantification of injury severity and accurate outcome prediction during acute care of the brain trauma patients. Identifying brain trauma biomarkers with strong prognostic values may highlight the pathophysiological pathways that best modulate neurological recovery and, therefore, enable researchers to focus their efforts into understanding these particular processes which may in turn allow identification of potential treatment points.

In reality, none of the proposed brain trauma biomarkers have been translated successfully into clinically useful prognostic tools despite growing number has been added to the literature over the last decade. These potential biomarkers are not evenly investigated, and the variations in methodology with sample types, sampling time points, and data analyses between different studies made it difficult to conclude from the exiting literature whether a particular biomarker is more superior at predicting brain trauma outcome.

By investigating multiple biomarkers simultaneously, we found that serum L-selectin level measured at day 1 post injury had the highest predictive value for unfavourable outcome after brain trauma. Its prognostic value was better than other inflammatory mediators such as interleukins and SICAM which is also an adhesion molecule. This finding highlights that although neuro-inflammation contributes toward neurological recovery after brain trauma, it is a heterogeneous process with different pathways commencing and subsiding at different time points after the initial brain trauma. Meaningful comparison and interpretation of the different biomarkers' outcome prognostic values will, therefore, need to be time defined to the initial injury.

The high outcome predictive value of serum L-selectin suggests that inflammatory processes involving L-selectin may be of particular importance to the modulation of neurological recovery following brain trauma. A recent adult stroke study has described patients with larger infarct volume to have significantly higher concentrations of plasma IL-6 and ICAM-1 than those with smaller infarct size [438]. In two other adult stroke studies, patients with different infarct sizes also have different concentrations of adhesion molecules [439, 440]. It is unclear whether the larger infarct size is caused by additional brain injury induced by the higher circulating cytokine concentrations post ischaemia, or it is the direct result of more severe initial ischaemia and the higher plasma cytokine levels are merely a reflection of the severity of the initial ischaemia.

Cellular adhesion molecules mediate leukocyte-endothelial interaction at the site of tissue injury which is vital for directing leukocytes into areas of acute inflammation. It has been shown that leukocytes and macrophages exacerbate brain injury following cerebral ischaemia by physically obstructing capillaries and reducing the blood flow (i.e. causing more ischaemic damage) to the injured brain [441, 442]. In addition, they also produce cytotoxic products once extravasated into the brain parenchyma [441, 442] which potentially may induce further brain insult. These may be mechanisms through which adhesion molecules modulate brain trauma outcome.

If the adhesion molecule mediated inflammatory response modulates brain trauma outcome by causing more brain insult from secondary ischaemic damage and cytotoxicity, its serum level should have no correlations with the burden of primary brain injury and secondary CPP insult. In addition, it may be out of proportion to these brain trauma outcome determinants. Of the 28 children with biomarker levels quantified, only 13 had CPP insult measured. The number of patients was, therefore, too small for further analysis to ascertain the relationships between adhesion molecule levels and CPP insult, which would warrant further investigations.

We additionally demonstrated for the first time that prognostic rules combining 2 biomarkers' serum levels had more superior prognostic value than individual markers when measured on day 1 post injury. This was not a surprising finding given the heterogenic nature of brain trauma pathophysiology. Importantly, we demonstrated that the choice of biomarkers within the prognostic rules influenced their prognostic values. Combining 2 biomarkers from the same mediator family was not as useful as using biomarkers from different mediator families for outcome prediction. We also demonstrated that although brain specific proteins measured on day 1 post injury were not as useful for outcome prognosis as the L-selectin, they dramatically improved a prognostic rule's outcome predictive value if either S100B or NSE was used as the screening marker component of the rule.

Assessing 8 biomarkers simultaneously after brain trauma provide more information than investigating individual markers. However, there are many more biomediators that regulate the cellular and molecular pathophysiological processes initiated by

brain trauma. Biomarkers that are not investigated in our current study may have higher modulating effects on brain trauma outcome than those of our chosen markers. Proteomic techniques allow simultaneous assessment of large number of molecules in bloody fluids and tissue specimens and may offer better understanding of the pathophysiological pathways after brain trauma.

7.5) ETHICAL CONSIDERATIONS

Clinical research is a vital link between basic science at the bench and clinical practice by the bedside but its success relies heavily upon patient availability and their participations. Conducting paediatric research is fought with difficulties since children are a vulnerable group of patients who may not have sufficient understanding to make an informed decision for themselves in research participation. Furthermore, the law relating to consenting in this area has never been clarified.

When seeking consent for medical treatment and procedures, children are the only people in British Law whereby consent may be given by an adult with parental responsibility who is usually the mother. The issue is more complex and confusing when teenagers are involved. At 16 years of age, every person is presumed able to give his/her own consent to treatment. However, before the age of 18 years, the courts or others with parental responsibility can override the refusal of a 'minor', even if this young person is deemed to be competent. In 1985, the House of Lords established that a person under the age of 16 who has sufficient understandings and intelligence may be competent to give consent to treatment by a clinician [443].

Similar principles have, therefore, been projected to consenting for research involving children which are detailed in the Principles of Paediatric Research as recommended by the Ethics Advisory Committee of the Royal College of Paediatrics and Child Health [444]. Unlike consenting for medical treatments, it is generally accepted that if a child can give a reasoned refusal to be involved in research, it is clear that this young person has sufficient understanding and it would be unwise to rely on parental consent alone in this situation. However, is it wise to include a child of sufficient understandings i.e. Gillick Competent who has given consent to take part in a research study but the parent refuses consent? Most paediatric research depends heavily upon parental co-operation, for example collection of follow-up data, and the research subjects often will not benefit from participating. Inclusion of such a child could possibly damage the trust of a parent and the future working relationship with the family without benefiting research development in medicine. It may, therefore, be best to respect the parent's wishes and to exclude the child's participation.

7.5.1) Consent to Participate in Research and Critically Ill Children

For research projects which aim to investigate emergency or critical care treatments, special considerations are required for consenting. This thesis relies completely on studies of critically ill brain injured children, most of whom were in a coma and were, therefore, incapable of giving consent to participate in the study. Most of their families were in great distress from the shock of learning that their beloved children

had sustained life threatening injuries, while others were involved in the same accident themselves and required ongoing treatment. Informed consent in these situations would, therefore, be impracticable or meaningless [445]. Recent evidence from Edinburgh demonstrated that at 18 months after enrolment into a clinical neonatal study, 12% of parents had no recollection of granting consent for their babies to participate in the study when the babies were critically ill, however, 83% of the parents from this study expressed their objections to the suggestion of abolishing the current consent process for trials approved by the local ethical committees [446]. In these situations, it is, therefore, paramount to conduct the consenting process as an ongoing phenomenon throughout the study.

For our study, initial consent was sought from the families with particular emphases on (i) the ability to change their minds regarding their participations in the study without affecting their children's treatment, and (ii) the reassurance that supports from the researchers would continue to help them better understand the study protocol and their involvements. This concept of obtaining continuing permission was particularly important because it removed some of the pressure from these families that were already in great distress and made the consent more valid. As a result of this approach, all suitable brain injured children have been recruited into the study and no family withdrew their involvements from the study. Furthermore, this improved the working relationship between the research team and the families facilitating the follow-up process which was a vital endpoint of this study.

7.5.2) Ethical Considerations in Research Involving Human Tissues

Thorough post-mortem examinations are crucial to clinical research by determining the cause of death in patients enrolled in clinical studies [447]. For brain trauma studies, researching post-mortem specimens is complicated by the fact that all brain trauma fatalities are required by law to be referred to the Procurator Fiscal in Scotland or the coroner in England and Wales who will then decide whether a Fiscal or Coronial post-mortem examination is required. The specimens collected at Fiscal or Coronial post-mortems are retained as part of the medical records and are purely for diagnostic purposes. In order to use these samples for research or teaching purposes, prior consent must be sought from the families. Furthermore, the organs, particularly the brain and spinal cords, at autopsy are often not in an ideal state for detail examination because they are too soft especially if they are immature or affected by pathology such as haemorrhage or ischaemia. To avoid losing vital information from examining these organs too early, retention usually occurs to allow fixation in formalin for 3 – 6 weeks which hardens the tissue to enable detail examinations. This also allows a second expert opinion to be obtained.

7.5.2.1) Organ Retention Scandals – The Alder Hey Inquiry

Public enquiries into the cardiac service at Bristol Royal Infirmary and the pathology service at the Royal Liverpool Children's Hospital has disclosed organ retention which has attracted much media and public attentions. The enquiry to investigate the removal, retention, and disposal of human organs and tissue following post-mortem

examinations at the Royal Liverpool Children's Hospital began in December 1999. The enquiry report was published in January 2001 highlighting several failures within the NHS Trust, the University of Liverpool, and the Liverpool Coroner's Office which resulted in the systematic removal of organs between 1988 and 1995 [378]. Coupled with the highly fuelled media furore, this report shocked the nation especially when disclosing the fact that in many of these cases (i) the organs were removed without consent, (ii) histological examinations were not subsequently carried out leaving preliminary post-mortem reports unfinished, and (iii) the organs and tissues were not used for education or research purposes [378].

The necessity to retain organs for further histological examinations after formalin fixation resulted in the development of archived diagnostic blocks and slides in many pathology departments across the country. These materials have been regarded as part of the medical records and it was understood that there was an obligation to keep the tissue for possible review. Their value in advancing medical teaching and research should not be underestimated. The long-term retention of organs has been more variable. In most centres, organs were disposed of following diagnostic examination. Occasionally organs of specific educational value were kept for teaching and research including displays in medical school museums, although the latter has decreased significantly in recent years.

The Human Tissue Act 1961 [448] has been used as a guideline to this practice and stated that informed consent is not required and one must merely established a 'lack of objection'. The Alder Hey Inquiry has demonstrated that this past practice is

overly paternalistic [378]. New guideline has been issued by the Royal College of Pathologists in 2000 [449]. Included in this document was a sample consent form for post-mortem examination which specifically seeks to establish not only a lack of objection to the necropsy, but also to the retention, disposal, and use of the materials removed at autopsy for medical research or teaching [449].

7.5.2.2) Impact of the Alder Hey Inquiry On Post-Mortem Rate, Manpower and Training in Histopathology

Post-mortem rate has fallen in general for many years [378, 447, 450, 451]. This may be partly explained by the well-recognised nationwide manpower crisis in histopathology and in particular academic histopathology [452, 453]. Worsening of this shortage has been observed since the eruption of the hostile media climate surrounding the Alder Hey inquiry which resulted in several pathologists, in particular paediatric pathologists leaving the specialty [454, 455] .

The negative publicity from these inquiries is likely to reduce the willingness of clinicians to seek consent for post-mortem examinations, and the readiness of the general public to grant consents which would further exacerbate the decline in autopsy numbers. Furthermore, fiscals and coroners have requested fewer necropsy over the recent years. The true impact of this rapid fall in autopsy rate may not be known for quite some time, but negative effects can be foretold in the determination of accurate statistics regarding causes of death, and training of post-graduate doctors in pathology and other specialty as well as undergraduate medical students. If the

present rate of decline continues, both clinical autopsies and pathologists sufficiently trained to perform them face extinction [456]. In addition, inadequate training in histopathology will have a knock on effect on the diagnostic services for other clinical specialties such as surgical oncology.

7.5.2.3) Impact of the Alder Hey Inquiry On Research

It is worrying that many published studies in modern medicine cited death as an endpoint of the studies but the cause of death in these cases were not confirmed by necropsy [457]. The impact of the decline in autopsy rates on academic medicine is already reflected in the significant reduction in the number of scientific papers presented from the UK to the European Paediatric Pathology Society in successive years since 1997 (57% in 1997, through 37%, 22%, 9% to 2% in 2001) [458]. Following the Alder Hey Inquiry era, it is apparent that the families of patients involved in clinical research are reluctant to give consent for post-mortem examinations and to use the necropsy materials for research as observed from our study. This is a particularly alarming trend because without research, medicine will stagnate and put the future patient care in jeopardy. Thus, for clinical research involving human tissue to continue, a programme of continuing education for the public is urgently required.

Several recently published reports and guidelines such as those produced by the Independent Review Group on the Retention of Organs at Post-mortem in Scotland (The McLean Review) [379] and the Medical Research Councils [380] are

attempting to clarify the position on conducting research on archival necropsy materials i.e. organs and tissue retained before December 2000. These reports and guidelines acknowledge the difficulties to seek retrospective consents from families for the use of archival tissues for ethically legitimate research or teaching purposes and recommend these material may be used for research provided that ethical approval has been granted by the local ethical committee for the proposed project and the specimens are used in an anonymous and unlinked manner [379, 380]. This has opened up an opportunity for our pathology study to adopt a modified aim and methods to investigate and classify the neuropathological patterns of fatal childhood TBI.

7.5.2.4) Post-Mortem and Research in the Future

The only way to ensure the survival of autopsy practice and research involving human tissue is by assisting bereaved families to reach an informed decision on post-mortem examinations, organ and tissue retention, and the use of these materials for research. This involves provision of information on what tissue samples may be taken at autopsies, why they are taken, and what would happen to them after the diagnostic procedure is completed. Equally important is the emphasis on what long-term information may be lost by not having a full post-mortem examination. Thus, paediatric clinicians, pathologists and neuropathologists must work together to ensure clinically relevant research will continue and to prevent stagnation and decline of academic medicine.

7.6) Limitations

This thesis has several limitations. Reduction in brain trauma cases is a welcome finding clinically, but limits the cohort size for research despite successful recruitment of all suitable patients during the study period. The small cohort size made it necessary to analyse APO E genetic polymorphisms' influence on CPP insult and outcome in dichotomies.

We had assessed the effect of a single gene polymorphism on CPP insult and outcome after brain trauma. With the complexity of the human genome, it would be important to investigate the effects of multiple genetic polymorphisms on brain trauma sequelae simultaneously.

Important prognostic rules using serum biomarker levels were formulated in this thesis to predict childhood brain trauma outcome but validation of these rules were not possible due to the paucity of the cohort size. The complex nature of secondary brain insult processes meant that potentially important prognostic information could be missed through investigations of multiple but selective biomarkers after brain trauma although it is still better than studying an individual marker at any one time.

7.7) Summary

This thesis has demonstrated that (1) cerebral ischaemia may be the common modulating pathway through which physiological factor and APO E genetic

polymorphisms affect brain trauma outcome; (2) neuro-inflammation may affect brain trauma recovery more than previously realised; and (3) combined quantification of neuro-inflammation and glial-neuronal damage provide better outcome prognostication than quantification of either of these components on their own.

CHAPTER 8: THESIS CONCLUSIONS AND FUTURE RESEARCH PROPOSAL

8.1) THESIS CONCLUSIONS

8.1.1) Summary of Significant Findings

Almost all previous childhood brain trauma studies failed to consider age-maturation effects of the intracranial haemodynamics and only measured individual excursion of pressure, or the duration of derangement. The novel cumulative pressure time index (PTI) quantifies the total burden of CPP insult by taking both the duration and magnitude of the insult into considerations [203, 204]. This quantification method is robust and allows insult comparison between different age groups [203, 204]. With PTI and grouping children into three age bands (aged 2 – 6 years, 7 – 10 years, and 11 – 15 years), age-related cerebral perfusion pressure (CPP) thresholds have been defined as 48, 54, and 58 mmHg for these age groups respectively [204]. We have also proven that the total burden of CPP insult (i.e. both the duration and magnitude) affects outcome after childhood brain trauma [204].

In addition, we demonstrated that children carrying the Apolipoprotein E (APO E) e4 allele with unfavourable outcome had 22 times less CPP insult than the non-e4 carriers after brain trauma, while the e3 homozygous with good recovery had 26 times more insult than the non-e3 homozygous [261]. This suggests that the APO E

genetic polymorphisms may differentially affect brain trauma patients' cerebral ischaemic tolerance [261].

Adhesion molecules may be more useful in diagnosing severe injury and predicting poor outcome after childhood brain trauma than brain specific proteins. Combining 2 serum biomarker levels in prognostic rules offered more superior outcome prediction than using the individual marker's levels. Prognostic rules that utilize the first day serum levels of 2 biomarkers from different mediator families may predict unfavourable outcome after brain trauma with 100% sensitivity and 96% specificity .

8.1.2) Conclusions

Cerebral ischaemia is an important determinant of childhood brain trauma which may be the common modulating pathway through which physiological and genetic factors affect brain trauma outcome. Neuro-inflammatory pathways involving adhesion molecules may be particularly important to neuronal recovery after brain trauma in children and their relationships with CPP insult warrants further investigation. Combined measurement of neuro-inflammation and glial-neuronal damage provide more superior outcome prognostication than quantification of either of these components on their own.

8.2) FUTURE RESEARCH PROPOSAL

8.2.1) Future Research Proposal Introduction

Associations between cerebral ischaemia and white matter damage are well established in adult brain trauma [373, 459-461] and stroke [462-464], and MRI imaging has demonstrated clearly that white matter damage occurs in childhood brain trauma and contributes significantly to outcome [465, 466]. The APO E genetic polymorphism may be important to white matter repair after brain trauma and cerebral ischaemia because of its potential allelic specific influence on the host's cerebral ischaemic tolerance [248, 261] and the involvement of its gene product in the brain's lipid transportation [144]. It is unknown whether the burden of CPP insult or APO E genotypes affect the degree of white matter damage differently after childhood brain trauma. In addition to its influence in the acute phase, APO E genotype also plays a major role in post-injury repair and recovery both in children and young adults after head injury [467] and in transgenic animals [468].

Neuroimaging is used to identify white matter damage but is limited by cost, access and provides only one time-point in a dynamic pathophysiology. Attempts to quantify brain damage using easily measurable and inexpensive individual serum biomarkers have been unsuccessful. We recently demonstrated that prognostic rules combining 2 biomarker serum levels measured on the first day after childhood brain trauma had more superior outcome predictive values than could be achieved using individual marker levels, and may be 100% sensitive and 96% specific for

unfavourable outcome at 6 month after the initial brain injury. A recent report described the utility of gel-free proteomics in identifying peripheral serum biomarkers of brain trauma [469]. It demonstrated that proteomics technique may be used to yield novel combinations of peripheral biomarkers with potentially higher informative value about disease characteristics than a single biomarker [469]. No study has investigated whether combination of proteins and their pattern behaviour have any correlation with the degree of white matter damage, the burden of CPP insult, and APO E genetic polymorphisms in childhood brain trauma.

The search for effective neuroprotective strategies has so far yielded only disappointing results in part because majority of the proposed novel strategies aim to modify grey matter damage [470]. Mild hypothermia (32 - 36°C) is a promising neuroprotectant in the laboratory, and recent evidence indicates that it may provide more effective neuro-protection than other proposed strategies by offering protection to both white matters and the grey matter after experimental non-impact brain injuries [471-474]. However, the precise mechanisms through which mild hypothermia affects brain trauma recovery remain unknown. Some investigators believe mild hypothermia confers its benefits solely by blocking all changes in signalling events that are detrimental to the ischaemic brain [475]. But others have demonstrated an increased neuroprotective protein synthesis with mild hypothermia after ischaemia [475-478]. If mild hypothermia is to be successfully translated into clinically effective therapy, understanding its effect on brain injuring mechanisms and white matter damage, and whether its actions differ in hosts with APO E and other genetic polymorphisms are essential.

Mild hypothermia has been demonstrated to be safe in clinical settings without life threatening complications that are associated with the historic use of prolonged deep hypothermia (30°C or less) [479-482]. Three recent independent clinical studies demonstrated an improved outcome among adults surviving cardio-pulmonary resuscitation [483-485]. In addition, reduced risks of death or disability were associated with selective head cooling [486] and whole body hypothermia [487] in neonatal hypoxic-ischaemic encephalopathy. Animal studies have suggested that a brief period of mild hypothermia is able to limit the extent of brain injury, improves mortality and morbidity after experimental brain trauma and cerebral ischaemia [488-491]. It is, however, unclear whether mild hypothermia alters brain trauma outcome in the clinical setting.

The reported benefits of mild hypothermia were associated with varying durations of hypothermia ranging from a few hours to 14 days [489-493]. The optimal hypothermia therapeutic duration is, however, unknown in both human and animal brain injury. It is also unclear whether mild hypothermia continues to offer its potential benefits if initiated several hours after the occurrence of brain trauma. This is a particularly important consideration when currently it may take up to 5 hours before brain trauma children receive definitive neuro-intensive care [494].

8.2.2) Hypotheses

We hypothesize that:

1. APO E e4 allelic children suffer more white matter damage after brain trauma than the non-e4 carriers because of their relative intolerance of cerebral ischaemia.
2. Pattern behaviour of proteins detected by gel-free proteomics are different in brain trauma children with varying degree of white matter damage, different burden of CPP insult, and different APO E genotypes.
3. Longer duration of mild hypothermia therapy, even if commenced after some delays, is more effective in reducing white matter damage than shorter treatment period in experimental brain trauma.
4. Animals expressing the different human APO E alleles respond differently to mild hypothermia therapy, have different patterns of white matter proteins, and different degree of white matter damage after brain injury.

8.2.3) Aims and Objectives

We aim to determine:

1. How APO E genetic polymorphisms and CPP insult influence white matter damage and outcome after childhood brain trauma.
2. The optimal therapeutic window and duration of mild hypothermia in experimental brain trauma.

3. Whether the APO E genetic polymorphisms affect the host's response to mild hypothermia and its mechanisms of action in experimental brain trauma.

8.2.4) Proposed Methodology

A 5-year project is proposed and will consist of a clinical study component, and a pre-clinical animal study component that will take place in Toronto, Canada, and Edinburgh, UK.

The clinical study will assess the influence of APO E genotypes and the total burden of CPP insult on the amount of white matter damage and outcome after brain trauma. In addition, serum proteomics will be investigated in these patients.

The pre-clinical animal study will be conducted in two stages over the five-year period. Phase 1 (2 of the 5 years) will be conducted at the Hospital for Sick Children (HSC), Toronto, Canada under the collaboration and supervision of Prof. James Hutchison. Phase 2 (3 of the 5 years) will be conducted at the University of Edinburgh, UK, under the supervision of Prof. James McCulloch and in collaboration with Dr. Karen Horsburgh. This part of the study will assess the effect of mild hypothermia on white matter damage after experimental brain trauma in wild type and APO E transgenic mice.

The unique hypothermia-brain trauma model in Toronto is not available in the UK. This collaboration is essential to the proposed project and will add significantly to the paediatric brain trauma literature. In addition, this collaboration will enable us to bring the hypothermia-brain trauma model back to Edinburgh. We then hope to optimize the model for application to APO E transgenic mice by capitalizing the expertise available in Edinburgh Neuroscience.

BIBLIOGRAPHY

1. Jennett B. Epidemiology of head injury. *J Neurol Neurosurg Psychiatry* 1996;60:362-369.
2. Walker PJ and Cass DT. Paediatric trauma: urban epidemiology and an analysis of methods for assessing the severity of trauma in 598 injured children. *Aust N Z J Surg* 1987;57:715-722.
3. Suominen P, Kivioja A, Ohman J, Korpela R, Rintala R and Olkkola KT. Severe and fatal childhood trauma. *Injury* 1998;29:425-430.
4. Jennett B. Epidemiology of head injury. *Arch Dis Child* 1998;78:403-406.
5. Taylor HG and Alden J. Age-related differences in outcomes following childhood brain insults: an introduction and overview. *J Int Neuropsychol Soc* 1997;3:555-567.
6. Tilford JM, Simpson PM, Yeh TS, Lensing S, Aitken ME, Green JW, *et al.* Variation in therapy and outcome for pediatric head trauma patients. *Crit Care Med* 2001;29:1056-1061.
7. Brookes M, MacMillan R, Cully S, Anderson E, Murray S, Mendelow AD, *et al.* Head injuries in accident and emergency departments. How different are children from adults? *J Epidemiol Community Health* 1990;44:147-151.
8. Morrison A, Stone DH, Redpath A, Campbell H and Norrie J. Trend analysis of socioeconomic differentials in deaths from injury in childhood in Scotland, 1981-95. *Bmj* 1999;318:567-568.
9. Williamson LM, Morrison A and Stone DH. Trends in head injury mortality among 0-14 year olds in Scotland (1986-95). *J Epidemiol Community Health* 2002;56:285-288.
10. Annegers JF, Grabow JD, Kurland LT and Laws ER, Jr. The incidence, causes, and secular trends of head trauma in Olmsted County, Minnesota, 1935-1974. *Neurology* 1980;30:912-919.
11. Kraus JF, Fife D, Cox P, Ramstein K and Conroy C. Incidence, severity, and external causes of pediatric brain injury. *Am J Dis Child* 1986;140:687-693.
12. Yager JY, Johnston B and Seshia SS. Coma scales in pediatric practice. *Am J Dis Child* 1990;144:1088-1091.
13. Gabella B, Hoffman RE, Marine WW and Stallones L. Urban and rural traumatic brain injuries in Colorado. *Ann Epidemiol* 1997;7:207-212.

14. Durkin MS, Laraque D, Lubman I and Barlow B. Epidemiology and prevention of traffic injuries to urban children and adolescents. *Pediatrics* 1999;103:e74.
15. Reid SR, Roesler JS, Gaichas AM and Tsai AK. The epidemiology of pediatric traumatic brain injury in Minnesota. *Arch Pediatr Adolesc Med* 2001;155:784-789.
16. Thurman D and Guerrero J. Trends in hospitalization associated with traumatic brain injury. *Jama* 1999;282:954-957.
17. Adekoya N, Thurman DJ, White DD and Webb KW. Surveillance for traumatic brain injury deaths--United States, 1989-1998. *MMWR Surveill Summ* 2002;51:1-14.
18. Levin HS, Aldrich EF, Saydjari C, Eisenberg HM, Foulkes MA, Bellefleur M, *et al.* Severe head injury in children: experience of the Traumatic Coma Data Bank. *Neurosurgery* 1992;31:435-443; discussion 443-434.
19. Tepas JJ, 3rd, DiScala C, Ramenofsky ML and Barlow B. Mortality and head injury: the pediatric perspective. *J Pediatr Surg* 1990;25:92-95; discussion 96.
20. Benoit R, Watts DD, Dwyer K, Kaufmann C and Fakhry S. Windows 99: a source of suburban pediatric trauma. *J Trauma* 2000;49:477-481; discussion 481-472.
21. Thurman D. Traumatic Brain Injury in Children and Youth as a Public Health Problem: An Overview. In: Langlois J. A., editor. *Traumatic Brain Injury in the United States: Assessing Outcomes in Children*. Atlanta, GA: Centers for Disease Control and Prevention, Department of Health and Human Services; 2000. p. 31-35.
22. Rantakallio P and von Wendt L. Trauma to the nervous system and its sequelae in a one-year birth cohort followed up to the age of 14 years. *J Epidemiol Community Health* 1985;39:353-356.
23. Hansen TB, Pless S and Gravers M. [Head injuries among children in the county of Ringkobing. 1. An analysis of hospital data]. *Ugeskr Laeger* 1991;153:2944-2946.
24. Arnarson EO and Halldorsson JG. Head trauma among children in Reykjavik. *Acta Paediatr* 1995;84:96-99.
25. Masson F, Thicoipe M, Aye P, Mokni T, Senjean P, Schmitt V, *et al.* Epidemiology of severe brain injuries: a prospective population-based study. *J Trauma* 2001;51:481-489.
26. Berney J, Favier J and Froidevaux AC. Paediatric head trauma: influence of age and sex. I. Epidemiology. *Childs Nerv Syst* 1994;10:509-516.

27. Masson F, Thicoipe M, Mokni T, Aye P, Erny P and Dabadie P. Epidemiology of traumatic comas: a prospective population-based study. *Brain Inj* 2003;17:279-293.
28. Teasdale G and Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet* 1974;2:81-84.
29. Toutant SM, Klauber MR, Marshall LF, Toole BM, Bowers SA, Seelig JM, *et al.* Absent or compressed basal cisterns on first CT scan: ominous predictors of outcome in severe head injury. *J Neurosurg* 1984;61:691-694.
30. Marshall LF, Marshall SB, Klauber MR and Van Berkum Clark M. A new classification of head injury based on computerized tomography. *Journal of Neurosurgery* 1991;75:S14-S20.
31. Marshall LF, Toole BM and Bowers SA. The National Traumatic Coma Data Bank. Part 2: Patients who talk and deteriorate: implications for treatment. *J Neurosurg* 1983;59:285-288.
32. Simpson D and Reilly P. Pediatric coma scale. *Lancet* 1982;2:450.
33. Raimondi AJ and Hirschauer J. Head injury in the infant and toddler. Coma scoring and outcome scale. *Childs Brain* 1984;11:12-35.
34. Jennett B, Teasdale G, Galbraith S, Pickard J, Grant H, Braakman R, *et al.* Severe head injuries in three countries. *J Neurol Neurosurg Psychiatry* 1977;40:291-298.
35. Huttenlocher PR. Reye's syndrome: relation of outcome to therapy. *J Pediatr* 1972;80:845-850.
36. Seshia SS, Seshia MM and Sachdeva RK. Coma in childhood. *Dev Med Child Neurol* 1977;19:614-628.
37. Morray JP, Tyler DC, Jones TK, Stuntz JT and Lemire RJ. Coma scale for use in brain-injured children. *Crit Care Med* 1984;12:1018-1020.
38. Gordon NS, Fois A, Jacobi G, Minns RA and Seshia SS. The management of the comatose child. *Neuropediatrics* 1983;14:3-5.
39. The Brain Trauma Foundation. The American Association of Neurological Surgeons. The Joint Section on Neurotrauma and Critical Care. Glasgow coma scale score. *J Neurotrauma* 2000;17:563-571.
40. Tatman A, Warren A, Williams A, Powell JE and Whitehouse W. Development of a modified paediatric coma scale in intensive care clinical practice. *Arch Dis Child* 1997;77:519-521.

41. Baker SP, O'Neill B, Haddon W, Jr. and Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974;14:187-196.
42. Boyd CR, Tolson MA and Copes WS. Evaluating trauma care: the TRISS method. Trauma Score and the Injury Severity Score. *J Trauma* 1987;27:370-378.
43. Tepas JJ, 3rd, Mollitt DL, Talbert JL and Bryant M. The pediatric trauma score as a predictor of injury severity in the injured child. *J Pediatr Surg* 1987;22:14-18.
44. Adelson PD, Clyde B, Kochanek PM, Wisniewski SR, Marion DW and Yonas H. Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. *Pediatr Neurosurg* 1997;26:200-207.
45. Crouchman M, Rossiter L, Colaco T and Forsyth R. A practical outcome scale for paediatric head injury. *Arch Dis Child* 2001;84:120-124.
46. Sharples PM, Storey A, Aynsley-Green A and Eyre JA. Causes of fatal childhood accidents involving head injury in northern region, 1979-86. *Bmj* 1990;301:1193-1197.
47. Chan BS, Walker PJ and Cass DT. Urban trauma: an analysis of 1,116 paediatric cases. *J Trauma* 1989;29:1540-1547.
48. Emanuelson I and v Wendt L. Epidemiology of traumatic brain injury in children and adolescents in south-western Sweden. *Acta Paediatr* 1997;86:730-735.
49. Andersson EH, Bjorklund R, Emanuelson I and Stalhammar D. Epidemiology of traumatic brain injury: a population based study in western Sweden. *Acta Neurol Scand* 2003;107:256-259.
50. Roberts I and Power C. Does the decline in child injury mortality vary by social class? A comparison of class specific mortality in 1981 and 1991. *Bmj* 1996;313:784-786.
51. Sharples PM. Head injury in children. In: Little R. A. and Ward Platt M. P., editors. *Injury in the young*: Cambridge University Press; 1998. p. 151-175.
52. Cicerone KD, Smith LC, Ellmo W, Mangel HR, Nelson P, Chase RF, *et al.* Neuropsychological rehabilitation of mild traumatic brain injury. *Brain Inj* 1996;10:277-286.
53. Roberts MA, Manshadi FF, Bushnell DL and Hines ME. Neurobehavioural dysfunction following mild traumatic brain injury in childhood: a case report with positive findings on positron emission tomography (PET). *Brain Inj* 1995;9:427-436.

54. Klonoff H, Clark C and Klonoff PS. Long-term outcome of head injuries: a 23 year follow up study of children with head injuries. *J Neurol Neurosurg Psychiatry* 1993;56:410-415.
55. Greenspan AI and MacKenzie EJ. Functional outcome after pediatric head injury. *Pediatrics* 1994;94:425-432.
56. Reilly PL, Graham DI, Adams JH and Jennett B. Patients with head injury who talk and die. *Lancet* 1975;2:375-377.
57. Rose J, Valtonen S and Jennett B. Avoidable factors contributing to death after head injury. *Br Med J* 1977;2:615-618.
58. Graham DI, Ford I, Adams JH, Doyle D, Teasdale GM, Lawrence AE, *et al.* Ischaemic brain damage is still common in fatal non-missile head injury. *J Neurol Neurosurg Psychiatry* 1989;52:346-350.
59. Sharples PM, Storey A, Aynsley-Green A and Eyre JA. Avoidable factors contributing to death of children with head injury. *Bmj* 1990;300:87-91.
60. Graham DI, Ford I, Adams JH, Doyle D, Lawrence AE, McLellan DR, *et al.* Fatal head injury in children. *J Clin Pathol* 1989;42:18-22.
61. Geddes JF, Hackshaw AK, Vowles GH, Nickols CD and Whitwell HL. Neuropathology of inflicted head injury in children. I. Patterns of brain damage. *Brain* 2001;124:1290-1298.
62. Geddes JF, Vowles GH, Hackshaw AK, Nickols CD, Scott IS and Whitwell HL. Neuropathology of inflicted head injury in children. II. Microscopic brain injury in infants. *Brain* 2001;124:1299-1306.
63. Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM, *et al.* The role of secondary brain injury in determining outcome from severe head injury. *J Trauma* 1993;34:216-222.
64. Price DJ and Murray A. The influence of hypoxia and hypotension on recovery from head injury. *Injury* 1972;3:218-224.
65. Miller JD, Butterworth JF, Gudeman SK, Faulkner JE, Choi SC, Selhorst JB, *et al.* Further experience in the management of severe head injury. *J Neurosurg* 1981;54:289-299.
66. Miller JD and Becker DP. Secondary insults to the injured brain. *J R Coll Surg Edinb* 1982;27:292-298.
67. Kohi YM, Mendelow AD, Teasdale GM and Allardice GM. Extracranial insults and outcome in patients with acute head injury--relationship to the Glasgow Coma Scale. *Injury* 1984;16:25-29.

68. Gentleman D and Jennett B. Hazards of inter-hospital transfer of comatose head-injured patients. *Lancet* 1981;2:853-854.
69. Miller JD, Sweet RC, Narayan R and Becker DP. Early insults to the injured brain. *Jama* 1978;240:439-442.
70. Miller JD, Becker DP, Ward JD, Sullivan HG, Adams WE and Rosner MJ. Significance of intracranial hypertension in severe head injury. *J Neurosurg* 1977;47:503-516.
71. Marmarou A, Anderson RL, Ward JD, Choi SC, Young HF, Eisenberg HM, *et al.* NINDS Traumatic Coma Data Bank: intracranial pressure monitoring methodology. *Journal of Neurosurgery* 1991;75:S21-S27.
72. Jones PA, Andrews PJ, Midgley S, Anderson SI, Piper IR, Tocher JL, *et al.* Measuring the burden of secondary insults in head-injured patients during intensive care. *J Neurosurg Anesthesiol* 1994;6:4-14.
73. Andrews PJ, Piper IR, Dearden NM and Miller JD. Secondary insults during intrahospital transport of head-injured patients. *Lancet* 1990;335:327-330.
74. Guidelines for the management of severe traumatic brain injury. *Journal of Neurotrauma* 2000;17:449-627.
75. Guidelines for the acute medical management of severe traumatic brain injury in infants, children and adolescents. *Pediatric Critical Care Medicine* 2003;4:S1 - 75.
76. Becker DP, Miller JD, Ward JD, Greenberg RP, Young HF and Sakalas R. The outcome from severe head injury with early diagnosis and intensive management. *J Neurosurg* 1977;47:491-502.
77. Graham DI and Adams JH. Ischaemic brain damage in fatal head injuries. *Lancet* 1971;1:265-266.
78. Graham DI, Adams JH and Doyle D. Ischaemic brain damage in fatal non-missile head injuries. *J Neurol Sci* 1978;39:213-234.
79. Graham DI, Lawrence AE, Adams JH, Doyle D and McLellan DR. Brain damage in fatal non-missile head injury without high intracranial pressure. *J Clin Pathol* 1988;41:34-37.
80. Murray GD, Teasdale GM, Braakman R, Cohadon F, Dearden M, Iannotti F, *et al.* The European Brain Injury Consortium survey of head injuries. *Acta Neurochir (Wien)* 1999;141:223-236.

81. Ghajar J, Hariri RJ, Narayan RK, Iacono LA, Firlik K and Patterson RH. Survey of critical care management of comatose, head-injured patients in the United States. *Crit Care Med* 1995;23:560-567.
82. Ghajar J. Traumatic brain injury. *Lancet* 2000;356:923-929.
83. Maas AI, Dearden M, Teasdale GM, Braakman R, Cohadon F, Iannotti F, *et al.* EBIC-guidelines for management of severe head injury in adults. European Brain Injury Consortium. *Acta Neurochir (Wien)* 1997;139:286-294.
84. Early management of head injury. *Scottish Intercollegiate Guidelines Network* 2000.
85. Muizelaar JP, Marmarou A, DeSalles AA, Ward JD, Zimmerman RS, Li Z, *et al.* Cerebral blood flow and metabolism in severely head-injured children. Part 1: Relationship with GCS score, outcome, ICP, and PVI. *J Neurosurg* 1989;71:63-71.
86. Sharples PM, Matthews DS and Eyre JA. Cerebral blood flow and metabolism in children with severe head injuries. Part 2: Cerebrovascular resistance and its determinants. *J Neurol Neurosurg Psychiatry* 1995;58:153-159.
87. Report of the Second Task Force on Blood Pressure Control in Children--1987. Task Force on Blood Pressure Control in Children. National Heart, Lung, and Blood Institute, Bethesda, Maryland. *Pediatrics* 1987;79:1-25.
88. Aldrich EF, Eisenberg HM, Saydjari C, Luerssen TG, Foulkes MA, Jane JA, *et al.* Diffuse brain swelling in severely head-injured children. A report from the NIH Traumatic Coma Data Bank. *J Neurosurg* 1992;76:450-454.
89. Sharples PM, Stuart AG, Matthews DS, Aynsley-Green A and Eyre JA. Cerebral blood flow and metabolism in children with severe head injury. Part 1: Relation to age, Glasgow coma score, outcome, intracranial pressure, and time after injury. *J Neurol Neurosurg Psychiatry* 1995;58:145-152.
90. Jackson S, Piper IR, Wagstaff A and *al. e.* Quantification of cerebral perfusion pressure (CPP) 'secondary insults' in children: a study of the effects of using different CPP thresholds. *British Journal of Neurosurgery* 1999;13:104.
91. Chambers IR, Treadwell L and Mendelow AD. The cause and incidence of secondary insults in severely head-injured adults and children. *Br J Neurosurg* 2000;14:424-431.
92. Jones PA, Andrews PJ, Easton VJ and Minns RA. Traumatic brain injury in childhood: intensive care time series data and outcome. *Br J Neurosurg* 2003;17:29-39.

93. Adams H, Mitchell DE, Graham DI and Doyle D. Diffuse brain damage of immediate impact type. Its relationship to 'primary brain-stem damage' in head injury. *Brain* 1977;100:489-502.
94. Bruce DA, Langfitt TW, Miller JD, Schutz H, Vapalahti MP, Stanek A, *et al.* Regional cerebral blood flow, intracranial pressure, and brain metabolism in comatose patients. *J Neurosurg* 1973;38:131-144.
95. Fujimoto T, Walker JTJ, Spatz M and *al. e.* Pathophysiologic aspects of ischaemic edema. In: Pappius H. M. and Feindel W., editors. Dynamics of brain edema. Heidelberg: Springer; 1976.
96. Erecinska M and Silver IA. Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* 1990;35:245-296.
97. Lipton SA and Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* 1994;330:613-622.
98. Choi DW. Ionic dependence of glutamate neurotoxicity. *J Neurosci* 1987;7:369-379.
99. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988;1:623-634.
100. Siesjo BK and Siesjo P. Mechanisms of secondary brain injury. *Eur J Anaesthesiol* 1996;13:247-268.
101. Lewen A, Matz P and Chan PH. Free radical pathways in CNS injury. *J Neurotrauma* 2000;17:871-890.
102. Siesjo BK, Bendek G, Koide T, Westerberg E and Wieloch T. Influence of acidosis on lipid peroxidation in brain tissues in vitro. *J Cereb Blood Flow Metab* 1985;5:253-258.
103. Babior BM, Curnutte JT and McMurrich BJ. The particulate superoxide-forming system from human neutrophils. Properties of the system and further evidence supporting its participation in the respiratory burst. *J Clin Invest* 1976;58:989-996.
104. Hall ED. The role of oxygen radicals in traumatic injury: clinical implications. *J Emerg Med* 1993;11 Suppl 1:31-36.
105. Chan PH, Schmidley JW, Fishman RA and Longar SM. Brain injury, edema, and vascular permeability changes induced by oxygen-derived free radicals. *Neurology* 1984;34:315-320.
106. McDonald RP, Horsburgh KJ, Graham DI and Nicoll JA. Mitochondrial DNA deletions in acute brain injury. *Neuroreport* 1999;10:1875-1878.

107. Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM and Floyd RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. *Proc Natl Acad Sci U S A* 1990;87:5144-5147.
108. Yakes FM and Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A* 1997;94:514-519.
109. Ott L, McClain CJ, Gillespie M and Young B. Cytokines and metabolic dysfunction after severe head injury. *J Neurotrauma* 1994;11:447-472.
110. Shohami E, Bass R, Wallach D, Yamin A and Gallily R. Inhibition of tumor necrosis factor alpha (TNFalpha) activity in rat brain is associated with cerebroprotection after closed head injury. *J Cereb Blood Flow Metab* 1996;16:378-384.
111. Stahel PF, Shohami E, Younis FM, Kariya K, Otto VI, Lenzlinger PM, *et al.* Experimental closed head injury: analysis of neurological outcome, blood-brain barrier dysfunction, intracranial neutrophil infiltration, and neuronal cell death in mice deficient in genes for pro-inflammatory cytokines. *J Cereb Blood Flow Metab* 2000;20:369-380.
112. Keeling KL, Hicks RR, Mahesh J, Billings BB and Kotwal GJ. Local neutrophil influx following lateral fluid-percussion brain injury in rats is associated with accumulation of complement activation fragments of the third component (C3) of the complement system. *J Neuroimmunol* 2000;105:20-30.
113. Whalen MJ, Carlos TM, Kochanek PM and Heineman S. Blood-brain barrier permeability, neutrophil accumulation and vascular adhesion molecule expression after controlled cortical impact in rats: a preliminary study. *Acta Neurochir Suppl* 1998;71:212-214.
114. Adelson PD, Whalen MJ, Kochanek PM, Robichaud P and Carlos TM. Blood brain barrier permeability and acute inflammation in two models of traumatic brain injury in the immature rat: a preliminary report. *Acta Neurochir Suppl* 1998;71:104-106.
115. Schlosshauer B. The blood-brain barrier: morphology, molecules, and neurothelin. *Bioessays* 1993;15:341-346.
116. Rebhun J, Madorsky JG and Glovsky MM. Proteins of the complement system and acute phase reactants in sera of patients with spinal cord injury. *Ann Allergy* 1991;66:335-338.
117. Kossmann T, Stahel PF, Morganti-Kossmann MC, Jones JL and Barnum SR. Elevated levels of the complement components C3 and factor B in ventricular

- cerebrospinal fluid of patients with traumatic brain injury. *J Neuroimmunol* 1997;73:63-69.
118. Baumann H and Gauldie J. The acute phase response. *Immunol Today* 1994;15:74-80.
119. Kossmann T, Hans VH, Imhof HG, Stocker R, Grob P, Trentz O, *et al.* Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. *Shock* 1995;4:311-317.
120. Young AB, Ott LG, Beard D, Dempsey RJ, Tibbs PA and McClain CJ. The acute-phase response of the brain-injured patient. *J Neurosurg* 1988;69:375-380.
121. McClain C, Cohen D, Phillips R, Ott L and Young B. Increased plasma and ventricular fluid interleukin-6 levels in patients with head injury. *J Lab Clin Med* 1991;118:225-231.
122. Bell MJ, Kochanek PM, Doughty LA, Carcillo JA, Adelson PD, Clark RS, *et al.* Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma* 1997;14:451-457.
123. Kalabalikis P, Papazoglou K, Gouriatis D, Papadopoulos N, Kardara M, Papageorgiou F, *et al.* Correlation between serum IL-6 and CRP levels and severity of head injury in children. *Intensive Care Med* 1999;25:288-292.
124. Kossmann T, Hans V, Imhof HG, Trentz O and Morganti-Kossmann MC. Interleukin-6 released in human cerebrospinal fluid following traumatic brain injury may trigger nerve growth factor production in astrocytes. *Brain Res* 1996;713:143-152.
125. Kossmann T, Stahel PF, Lenzlinger PM, Redl H, Dubs RW, Trentz O, *et al.* Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J Cereb Blood Flow Metab* 1997;17:280-289.
126. Whalen MJ, Carlos TM, Kochanek PM, Wisniewski SR, Bell MJ, Clark RS, *et al.* Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. *Crit Care Med* 2000;28:929-934.
127. Gallinaro R, Cheadle WG, Applegate K and Polk HC, Jr. The role of the complement system in trauma and infection. *Surg Gynecol Obstet* 1992;174:435-440.
128. Heideman M. The role of complement in trauma. *Acta Chir Scand Suppl* 1985;522:233-244.
129. Kapur MM, Jain P and Gidh M. The effect of trauma on serum C3 activation and its correlation with injury severity score in man. *J Trauma* 1986;26:464-466.

130. Hugli TE. Structure and function of the anaphylatoxins. *Springer Semin Immunopathol* 1984;7:193-219.
131. Hugli TE. Structure and function of C3a anaphylatoxin. *Curr Top Microbiol Immunol* 1990;153:181-208.
132. Gerard C and Gerard NP. C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu Rev Immunol* 1994;12:775-808.
133. Furie MB and Randolph GJ. Chemokines and tissue injury. *Am J Pathol* 1995;146:1287-1301.
134. Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989;320:365-376.
135. Bellander BM, von Holst H, Fredman P and Svensson M. Activation of the complement cascade and increase of clusterin in the brain following a cortical contusion in the adult rat. *J Neurosurg* 1996;85:468-475.
136. Farkas I, Baranyi L, Okada N, Liposits Z and Okada H. A complement C5a fragment peptide causes apoptosis in TGW neuroblastoma cells. *Neurobiol Aging* 1996;17:S183.
137. Rink A, Fung KM, Trojanowski JQ, Lee VM, Neugebauer E and McIntosh TK. Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *Am J Pathol* 1995;147:1575-1583.
138. Yakovlev AG, Knobloch SM, Fan L, Fox GB, Goodnight R and Faden AI. Activation of CPP32-like caspases contributes to neuronal apoptosis and neurological dysfunction after traumatic brain injury. *J Neurosci* 1997;17:7415-7424.
139. Ng I, Yeo TT, Tang WY, Soong R, Ng PY and Smith DR. Apoptosis occurs after cerebral contusions in humans. *Neurosurgery* 2000;46:949-956.
140. Hausmann R, Biermann T, Wiest I, Tubel J and Betz P. Neuronal apoptosis following human brain injury. *Int J Legal Med* 2004;118:32-36.
141. Teasdale GM, Nicoll JA, Murray G and Fiddes M. Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 1997;350:1069-1071.
142. Lichtman SW, Seliger G, Tycko B and Marder K. Apolipoprotein E and functional recovery from brain injury following postacute rehabilitation. *Neurology* 2000;55:1536-1539.

143. Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J, *et al.* Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* 1999;52:244-248.
144. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622-630.
145. Kraus JF and McArthur DL. Epidemiologic aspects of brain injury. *Neurol Clin* 1996;14:435-450.
146. Hillier SL, Hiller JE and Metzger J. Epidemiology of traumatic brain injury in South Australia. *Brain Inj* 1997;11:649-659.
147. Report of special Task Force. Guidelines for the determination of brain death in children. American Academy of Pediatrics Task Force on Brain Death in Children. *Pediatrics* 1987;80:298-300.
148. Cantais E, Paut O, Giorgi R, Viard L and Camboulives J. Evaluating the prognosis of multiple, severely traumatized children in the intensive care unit. *Intensive Care Med* 2001;27:1511-1517.
149. Cassidy LD, Potoka DA, Adelson PD and Ford HR. Development of a novel method to predict disability after head trauma in children. *J Pediatr Surg* 2003;38:482-485.
150. Eftekhari B, Mohammad K, Ardebili HE, Ghodsi M and Ketabchi E. Comparison of artificial neural network and logistic regression models for prediction of mortality in head trauma based on initial clinical data. *BMC Med Inform Decis Mak* 2005;5:3.
151. Facco E, Zuccarello M, Pittoni G, Zanardi L, Chiaranda M, Davia G, *et al.* Early outcome prediction in severe head injury: comparison between children and adults. *Childs Nerv Syst* 1986;2:67-71.
152. McDonald CM, Jaffe KM, Fay GC, Polissar NL, Martin KM, Liao S, *et al.* Comparison of indices of traumatic brain injury severity as predictors of neurobehavioral outcome in children. *Arch Phys Med Rehabil* 1994;75:328-337.
153. Ong L, Selladurai BM, Dhillon MK, Atan M and Lye MS. The prognostic value of the Glasgow Coma Scale, hypoxia and computerised tomography in outcome prediction of pediatric head injury. *Pediatr Neurosurg* 1996;24:285-291.
154. Ono J, Yamaura A, Kubota M, Okimura Y and Isobe K. Outcome prediction in severe head injury: analyses of clinical prognostic factors. *J Clin Neurosci* 2001;8:120-123.

155. Selladurai BM, Jayakumar R, Tan YY and Low HC. Outcome prediction in early management of severe head injury: an experience in Malaysia. *Br J Neurosurg* 1992;6:549-557.
156. Young B, Rapp RP, Norton JA, Haack D, Tibbs PA and Bean JR. Early prediction of outcome in head-injured patients. *J Neurosurg* 1981;54:300-303.
157. Grewal M and Sutcliffe AJ. Early prediction of outcome following head injury in children: an assessment of the value of Glasgow Coma Scale score trend and abnormal plantar and pupillary light reflexes. *J Pediatr Surg* 1991;26:1161-1163.
158. Fearnside MR, Cook RJ, McDougall P and McNeil RJ. The Westmead Head Injury Project outcome in severe head injury. A comparative analysis of pre-hospital, clinical and CT variables. *Br J Neurosurg* 1993;7:267-279.
159. Signorini DF, Andrews PJ, Jones PA, Wardlaw JM and Miller JD. Predicting survival using simple clinical variables: a case study in traumatic brain injury. *J Neurol Neurosurg Psychiatry* 1999;66:20-25.
160. Griffith RL and Becker DP. Physiological monitoring of the head injury patient. *Adv Neurol* 1979;22:51-72.
161. Sklar FH, Beyer CW, Jr. and Clark WK. Physiological features of the pressure-volume function of brain elasticity in man. *J Neurosurg* 1980;53:166-172.
162. Gaab MR, Ungersbock K and Hufenbeck B. Evaluation of ICP by computerized bedside monitoring: methods and clinical significance. *Neurol Res* 1986;8:44-52.
163. Skogseid IM, Nordby HK, Urdal P, Paus E and Lilleaas F. Increased serum creatine kinase BB and neuron specific enolase following head injury indicates brain damage. *Acta Neurochir (Wien)* 1992;115:106-111.
164. Yamazaki Y, Yada K, Morii S, Kitahara T and Ohwada T. Diagnostic significance of serum neuron-specific enolase and myelin basic protein assay in patients with acute head injury. *Surg Neurol* 1995;43:267-270; discussion 270-261.
165. Ergun R, Bostanci U, Akdemir G, Beskonakli E, Kaptanoglu E, Gursoy F, *et al.* Prognostic value of serum neuron-specific enolase levels after head injury. *Neurol Res* 1998;20:418-420.
166. Woertgen C, Rothoerl RD, Holzschuh M, Metz C and Brawanski A. Comparison of serial S-100 and NSE serum measurements after severe head injury. *Acta Neurochir (Wien)* 1997;139:1161-1164; discussion 1165.
167. Marangos PJ and Schmechel DE. Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Annu Rev Neurosci* 1987;10:269-295.

168. Rothoerl RD, Woertgen C, Holzschuh M, Metz C and Brawanski A. S-100 serum levels after minor and major head injury. *J Trauma* 1998;45:765-767.
169. McKeating EG, Andrews PJ and Mascia L. The relationship of soluble adhesion molecule concentrations in systemic and jugular venous serum to injury severity and outcome after traumatic brain injury. *Anesth Analg* 1998;86:759-765.
170. Whalen MJ, Carlos TM, Kochanek PM, Wisniewski SR, Bell MJ, Carcillo JA, *et al.* Soluble adhesion molecules in CSF are increased in children with severe head injury. *J Neurotrauma* 1998;15:777-787.
171. McKeating EG, Andrews PJ, Signorini DF and Mascia L. Transcranial cytokine gradients in patients requiring intensive care after acute brain injury. *Br J Anaesth* 1997;78:520-523.
172. Csuka E, Morganti-Kossmann MC, Lenzlinger PM, Joller H, Trentz O and Kossmann T. IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol* 1999;101:211-221.
173. Morganti-Kossmann MC, Hans VH, Lenzlinger PM, Dubs R, Ludwig E, Trentz O, *et al.* TGF-beta is elevated in the CSF of patients with severe traumatic brain injuries and parallels blood-brain barrier function. *J Neurotrauma* 1999;16:617-628.
174. Sherwood ER and Prough DS. Interleukin-8, neuroinflammation, and secondary brain injury. *Crit Care Med* 2000;28:1221-1223.
175. Maier B, Schwerdtfeger K, Mautes A, Holanda M, Muller M, Steudel WI, *et al.* Differential release of interleukines 6, 8, and 10 in cerebrospinal fluid and plasma after traumatic brain injury. *Shock* 2001;15:421-426.
176. Kushi H, Saito T, Makino K and Hayashi N. IL-8 is a key mediator of neuroinflammation in severe traumatic brain injuries. *Acta Neurochir Suppl* 2003;86:347-350.
177. Guyer B, Martin JA, MacDorman MF, Anderson RN and Strobino DM. Annual summary of vital statistics--1996. *Pediatrics* 1997;100:905-918.
178. Minns RA. Infectious and parainfectious encephalopathies. london: Mac Keith Press; 1991.
179. Pigula FA, Wald SL, Shackford SR and Vane DW. The effect of hypotension and hypoxia on children with severe head injuries. *J Pediatr Surg* 1993;28:310-314; discussion 315-316.

180. Vander AJ, Sherman JH and Luciano DS. Regulation of organic, metabolic and energy balance. In: Human Physiology. The mechanisms of body function. 2nd edition ed. New York: McGraw-Hill Inc; 1975. p. 391-429.
181. Update on the 1987 Task Force Report on High Blood Pressure in Children and Adolescents: a working group report from the National High Blood Pressure Education Program. National High Blood Pressure Education Program Working Group on Hypertension Control in Children and Adolescents. *Pediatrics* 1996;98:649-658.
182. Rosner B, Prineas RJ, Loggie JM and Daniels SR. Blood pressure nomograms for children and adolescents, by height, sex, and age, in the United States. *J Pediatr* 1993;123:871-886.
183. Donmall MC. Anthropometric and blood pressure studies in children from Shetland: Reliability of measurement and the use of height-weight indices. *Acta Paediatr Scand Suppl* 1985;74:23-35.
184. Moront ML, Williams JA, Eichelberger MR and Wilkinson JD. The injured child. An approach to care. *Pediatr Clin North Am* 1994;41:1201-1226.
185. Mann N and Punt J. Intracranial pressure monitoring in children. *Care Critically III* 1999;2:143-146.
186. Welch K. The intracranial pressure in infants. *J Neurosurg* 1980;52:693-699.
187. Lam WH. Mechanism and management of paediatric head injury. *Care Critically III* 1999;15:95-98.
188. Skippen P, Seear M, Poskitt K, Kestle J, Cochrane D, Annich G, *et al.* Effect of hyperventilation on regional cerebral blood flow in head-injured children. *Crit Care Med* 1997;25:1402-1409.
189. James HE. The management of head injury in children. *Neurosurg Quart* 1993;3:272-282.
190. Shapiro K and Smith LP. Special consideration for the paediatric age group. In: Cooper P. R., editor. Head Injury. Baltimore: Williams and Wilkins; 1993. p. 427-457.
191. Czosnyka M, Smielewski P, Kirkpatrick P, Menon DK and Pickard JD. Monitoring of cerebral autoregulation in head-injured patients. *Stroke* 1996;27:1829-1834.
192. Andrews PJ. What is the optimal perfusion pressure after brain injury - a review of the evidence with an emphasis on arterial pressure. *Acta Anaesthesiol Scand* 1995;105:112-114.

193. Guidelines for cerebral perfusion pressure. Brain Trauma Foundation. *J Neurotrauma* 1996;13:693-697.
194. Chan KH, Miller JD, Dearden NM, Andrews PJ and Midgley S. The effect of changes in cerebral perfusion pressure upon middle cerebral artery blood flow velocity and jugular bulb venous oxygen saturation after severe brain injury. *J Neurosurg* 1992;77:55-61.
195. Chan KH, Dearden NM, Miller JD, Andrews PJ and Midgley S. Multimodality monitoring as a guide to treatment of intracranial hypertension after severe brain injury. *Neurosurgery* 1993;32:547-552; discussion 552-543.
196. Hosmer DW and Lemeshow S. Applied logistic regression. New York: John Wiley & Sons Inc; 1989.
197. Abbate B, Donati P and Cagnoni G. [Head injuries in children. Considerations on 3,715 consecutive cases]. *Minerva Pediatr* 2000;52:623-628.
198. Minns RA and Lo TYM. Head and neck injuries. In: Keeling J. W. and Busuttill A., editors. Paediatric forensic medicine and pathology. London: Edward Arnold; 2008 (In press).
199. Cortbus F, Jones PA, Miller JD, Piper IR and Tocher JL. Cause, distribution and significance of episodes of reduced cerebral perfusion pressure following head injury. *Acta Neurochir (Wien)* 1994;130:117-124.
200. Mayer TA and Walker ML. Pediatric head injury: the critical role of the emergency physician. *Ann Emerg Med* 1985;14:1178-1184.
201. Pfenninger J, Kaiser G, Lutschg J and Sutter M. Treatment and outcome of the severely head injured child. *Intensive Care Med* 1983;9:13-16.
202. Esparza J, J MP, Sarabia M, Yuste JA, Roger R and Lamas E. Outcome in children with severe head injuries. *Childs Nerv Syst* 1985;1:109-114.
203. Jones PA, Chambers IR, Lo TY, Andrews PJ, Chaudhry W, Clark A, *et al.* Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure-time index. *Acta Neurochir Suppl* 2005;95:29-32.
204. Chambers IR, Jones PA, Lo TY, Forsyth RJ, Fulton B, Andrews PJ, *et al.* Critical thresholds of intracranial pressure and cerebral perfusion pressure related to age in paediatric head injury. *J Neurol Neurosurg Psychiatry* 2006;77:234-240.
205. Altman DG and Bland JM. Diagnostic tests 3: receiver operating characteristic plots. *Bmj* 1994;309:188.
206. Hanley JA and McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36.

207. Chambers IR, Stobbert L, Jones PA, Kirkham FJ, Marsh M, Mendelow AD, *et al.* Age-related differences in intracranial pressure and cerebral perfusion pressure in the first 6 hours of monitoring after children's head injury: association with outcome. *Childs Nerv Syst* 2005;21:195-199.
208. Luerssen TG, Klauber MR and Marshall LF. Outcome from head injury related to patient's age. A longitudinal prospective study of adult and pediatric head injury. *J Neurosurg* 1988;68:409-416.
209. Michaud LJ, Rivara FP, Grady MS and Reay DT. Predictors of survival and severity of disability after severe brain injury in children. *Neurosurgery* 1992;31:254-264.
210. Coates BM, Vavilala MS, Mack CD, Muangman S, Suz P, Sharar SR, *et al.* Influence of definition and location of hypotension on outcome following severe pediatric traumatic brain injury. *Crit Care Med* 2005;33:2645-2650.
211. Kokoska ER, Smith GS, Pittman T and Weber TR. Early hypotension worsens neurological outcome in pediatric patients with moderately severe head trauma. *J Pediatr Surg* 1998;33:333-338.
212. Kanter RK, Carroll JB and Post EM. Association of arterial hypertension with poor outcome in children with acute brain injury. *Clin Pediatr (Phila)* 1985;24:320-323.
213. Sahuquillo J, Amoros S, Santos A, Poca MA, Valenzuela H, Baguena M, *et al.* False autoregulation (pseudoautoregulation) in patients with severe head injury. Its importance in CPP management. *Acta Neurochir Suppl* 2000;76:485-490.
214. White JRM, Farukhi Z, Bull C, Christensen J, Gordon T, Paidas C, *et al.* Predictors of outcome in severely head-injured children. *Crit Care Med* 2001;29:534-540.
215. Chiaretti A, De Benedictis R, Della Corte F, Piastra M, Viola L, Polidori G, *et al.* The impact of initial management on the outcome of children with severe head injury. *Childs Nerv Syst* 2002;18:54-60.
216. Chiaretti A, Piastra M, Pulitano S, Pietrini D, De Rosa G, Barbaro R, *et al.* Prognostic factors and outcome of children with severe head injury: an 8-year experience. *Childs Nerv Syst* 2002;18:129-136.
217. Michaud LJ, Duhaime AC and Batshaw ML. Traumatic brain injury in children. *Pediatr Clin North Am* 1993;40:553-565.
218. Zauner A, Bullock R, Di X and Young HF. Brain oxygen, CO₂, pH, and temperature monitoring: evaluation in the feline brain. *Neurosurgery* 1995;37:1168-1176; discussion 1176-1167.

219. Menzel M, Rieger A, Roth S, Soukup J, Furka I, Miko I, *et al.* Comparison between continuous brain tissue pO₂, pCO₂, pH, and temperature and simultaneous cerebrovenous measurement using a multisensor probe in a porcine intracranial pressure model. *J Neurotrauma* 1998;15:265-276.
220. Zauner A, Doppenberg E, Woodward JJ, Allen C, Jebraili S, Young HF, *et al.* Multiparametric continuous monitoring of brain metabolism and substrate delivery in neurosurgical patients. *Neurol Res* 1997;19:265-273.
221. Charbel FT, Du X, Hoffman WE and Ausman JI. Brain tissue PO₂(2), PCO₂(2), and pH during cerebral vasospasm. *Surg Neurol* 2000;54:432-437; discussion 438.
222. Gopinath SP, Robertson CS, Contant CF, Hayes C, Feldman Z, Narayan RK, *et al.* Jugular venous desaturation and outcome after head injury. *J Neurol Neurosurg Psychiatry* 1994;57:717-723.
223. Aaslid R, Markwalder TM and Nornes H. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg* 1982;57:769-774.
224. Weber M, Grolimund P and Seiler RW. Evaluation of posttraumatic cerebral blood flow velocities by transcranial Doppler ultrasonography. *Neurosurgery* 1990;27:106-112.
225. Martin NA, Doberstein C, Zane C, Caron MJ, Thomas K and Becker DP. Posttraumatic cerebral arterial spasm: transcranial Doppler ultrasound, cerebral blood flow, and angiographic findings. *J Neurosurg* 1992;77:575-583.
226. Chan KH, Miller JD and Dearden NM. Intracranial blood flow velocity after head injury: relationship to severity of injury, time, neurological status and outcome. *J Neurol Neurosurg Psychiatry* 1992;55:787-791.
227. Ungerstedt U. Microdialysis--principles and applications for studies in animals and man. *J Intern Med* 1991;230:365-373.
228. Hillered L and Persson L. Neurochemical monitoring of the acutely injured human brain. *Scand J Clin Lab Invest Suppl* 1999;229:9-18.
229. Hillered L, Persson L, Ponten U and Ungerstedt U. Neurometabolic monitoring of the ischaemic human brain using microdialysis. *Acta Neurochir (Wien)* 1990;102:91-97.
230. Hillered L, Valtysson J, Enblad P and Persson L. Interstitial glycerol as a marker for membrane phospholipid degradation in the acutely injured human brain. *J Neurol Neurosurg Psychiatry* 1998;64:486-491.

231. Goodman JC, Valadka AB, Gopinath SP, Uzura M and Robertson CS. Extracellular lactate and glucose alterations in the brain after head injury measured by microdialysis. *Crit Care Med* 1999;27:1965-1973.
232. Hovda DA, Lee SM, Smith ML, Von Stuck S, Bergsneider M, Kelly D, *et al.* The neurochemical and metabolic cascade following brain injury: moving from animal models to man. *J Neurotrauma* 1995;12:903-906.
233. Nilsson OG, Brandt L, Ungerstedt U and Saveland H. Bedside detection of brain ischemia using intracerebral microdialysis: subarachnoid hemorrhage and delayed ischemic deterioration. *Neurosurgery* 1999;45:1176-1184; discussion 1184-1175.
234. Bullock R, Zauner A, Woodward JJ, Myseros J, Choi SC, Ward JD, *et al.* Factors affecting excitatory amino acid release following severe human head injury. *J Neurosurg* 1998;89:507-518.
235. Bell MJ, Robertson CS, Kochanek PM, Goodman JC, Gopinath SP, Carcillo JA, *et al.* Interstitial brain adenosine and xanthine increase during jugular venous oxygen desaturations in humans after traumatic brain injury. *Crit Care Med* 2001;29:399-404.
236. Reilly PL, Simpson DA, Sprod R and Thomas L. Assessing the conscious level in infants and young children: a paediatric version of the Glasgow Coma Scale. *Childs Nerv Syst* 1988;4:30-33.
237. Baker SP and O'Neill B. The injury severity score: an update. *J Trauma* 1976;16:882-885.
238. Howells TP. Pattern recognition in physiological time series data using Bayesian Neural Networks. In. Edinburgh: University of Edinburgh; 2002.
239. McQuatt A, Andrews PJ, Sleeman D, Corruble V and Jones PA. The analysis of head injury data using decision tree techniques. *Lecture Notes in Artificial Intelligence* 1999;1620:336-345.
240. Signorini DF, Andrews PJ, Jones PA, Wardlaw JM and Miller JD. Adding insult to injury: the prognostic value of early secondary insults for survival after traumatic brain injury. *J Neurol Neurosurg Psychiatry* 1999;66:26-31.
241. Stocchetti N, Rossi S, Buzzi F, Mattioli C, Paparella A and Colombo A. Intracranial hypertension in head injury: management and results. *Intensive Care Med* 1999;25:371-376.
242. Downard C, Hulka F, Mullins RJ, Piatt J, Chesnut R, Quint P, *et al.* Relationship of cerebral perfusion pressure and survival in pediatric brain-injured patients. *J Trauma* 2000;49:654-658; discussion 658-659.

243. Jackson S, Piper IR, Wagstaff A and Souter M. A study of the effects of using different cerebral perfusion pressure (CPP) thresholds to quantify CPP "secondary insults" in children. *Acta Neurochir Suppl* 2000;76:453-456.
244. Chambers IR, Treadwell L and Mendelow AD. Determination of threshold levels of cerebral perfusion pressure and intracranial pressure in severe head injury by using receiver-operating characteristic curves: an observational study in 291 patients. *J Neurosurg* 2001;94:412-416.
245. Jones PA, Minns RA, Lo TY, Andrews PJ, Taylor GS and Ali S. Graphical display of variability and inter-relationships of pressure signals in children with traumatic brain injury. *Physiol Meas* 2003;24:201-211.
246. Marmarou A, Anderson RL, Ward JD, Choi SC, Young HF, Eisenberg HM, *et al.* Impact of ICP instability and hypotension on outcome in patients with severe head injury. *Journal of Neurosurgery* 1991;75:S59-S66.
247. Utermann G, Langenbeck U, Beisiegel U and Weber W. Genetics of the apolipoprotein E system in man. *Am J Hum Genet* 1980;32:339-347.
248. Horsburgh K, McCulloch J, Nilsen M, Roses AD and Nicoll JA. Increased neuronal damage and apoE immunoreactivity in human apolipoprotein E, E4 isoform-specific, transgenic mice after global cerebral ischaemia. *Eur J Neurosci* 2000;12:4309-4317.
249. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC, Jr., *et al.* Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* 1994;7:180-184.
250. Alberts MJ, Graffagnino C, McClenny C, DeLong D, Strittmatter W, Saunders AM, *et al.* ApoE genotype and survival from intracerebral haemorrhage. *Lancet* 1995;346:575.
251. Sabo T, Lomnitski L, Nyska A, Beni S, Maronpot RR, Shohami E, *et al.* Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. *Neuroscience* 2000;101:879-884.
252. Buttini M, Akeefe H, Lin C, Mahley RW, Pitas RE, Wyss-Coray T, *et al.* Dominant negative effects of apolipoprotein E4 revealed in transgenic models of neurodegenerative disease. *Neuroscience* 2000;97:207-210.
253. Colton CA, Needham LK, Brown C, Cook D, Rasheed K, Burke JR, *et al.* APOE genotype-specific differences in human and mouse macrophage nitric oxide production. *J Neuroimmunol* 2004;147:62-67.

254. Aono M, Lee Y, Grant ER, Zivin RA, Pearlstein RD, Warner DS, *et al.* Apolipoprotein E protects against NMDA excitotoxicity. *Neurobiol Dis* 2002;11:214-220.
255. Mazur-Kolecka B, Dickson D and Frackowiak J. Induction of vascular amyloidosis-beta by oxidative stress depends on APOE genotype. *Neurobiol Aging* 2006;27:804-814.
256. Mattson MP. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol Rev* 1997;77:1081-1132.
257. Cumming AM and Robertson FW. Polymorphism at the apoprotein-E locus in relation to risk of coronary disease. *Clin Genet* 1984;25:310-313.
258. Millar K, Nicoll JA, Thornhill S, Murray GD and Teasdale GM. Long term neuropsychological outcome after head injury: relation to APOE genotype. *J Neurol Neurosurg Psychiatry* 2003;74:1047-1052.
259. Chiang MF, Chang JG and Hu CJ. Association between apolipoprotein E genotype and outcome of traumatic brain injury. *Acta Neurochir (Wien)* 2003;145:649-653; discussion 653-644.
260. Alexander S, Kerr ME, Kim Y, Kamboh MI, Beers SR and Conley YP. Apolipoprotein E4 allele presence and functional outcome after severe traumatic brain injury. *J Neurotrauma* 2007;24:790-797.
261. Lo TY, Jones PA, Chambers IR, Beattie TF, Forsyth R, Mendelow AD, *et al.* Modulating effect of apolipoprotein E polymorphisms on secondary brain insult and outcome after childhood brain trauma. *Childs Nerv Syst* 2009;25:47-54.
262. Davignon J, Gregg RE and Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 1988;8:1-21.
263. Becher JC, Bell JE, McIntosh N and Keeling JW. Distribution of apolipoprotein E alleles in a Scottish healthy newborn population. *Biol Neonate* 2005;88:164-167.
264. Smith C, Graham DI, Murray LS, Stewart J and Nicoll JA. Association of APOE e4 and cerebrovascular pathology in traumatic brain injury. *J Neurol Neurosurg Psychiatry* 2006;77:363-366.
265. Guo L, LaDu MJ and Van Eldik LJ. A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci* 2004;23:205-212.
266. Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, *et al.* APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003;278:48529-48533.

267. Persson L, Hardemark HG, Gustafsson J, Rundstrom G, Mendel-Hartvig I, Esscher T, *et al.* S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke* 1987;18:911-918.
268. Elting JW, de Jager AE, Teelken AW, Schaaf MJ, Maurits NM, van der Naalt J, *et al.* Comparison of serum S-100 protein levels following stroke and traumatic brain injury. *J Neurol Sci* 2000;181:104-110.
269. Karkela J, Bock E and Kaukinen S. CSF and serum brain-specific creatine kinase isoenzyme (CK-BB), neuron-specific enolase (NSE) and neural cell adhesion molecule (NCAM) as prognostic markers for hypoxic brain injury after cardiac arrest in man. *J Neurol Sci* 1993;116:100-109.
270. Vazquez MD, Sanchez-Rodriguez F, Osuna E, Diaz J, Cox DE, Perez-Carceles MD, *et al.* Creatine kinase BB and neuron-specific enolase in cerebrospinal fluid in the diagnosis of brain insult. *Am J Forensic Med Pathol* 1995;16:210-214.
271. Endo T, Tanaka T, Isobe T, Kasai H, Okuyama T and Hidaka H. Calcium-dependent affinity chromatography of S-100 and calmodulin on calmodulin antagonist-coupled Sepharose. *J Biol Chem* 1981;256:12485-12489.
272. Isobe T, Takahashi K and Okuyama T. S100a0 (alpha alpha) protein is present in neurons of the central and peripheral nervous system. *J Neurochem* 1984;43:1494-1496.
273. Jackson RG, Sales KM, Samra GS and Strunin L. Extra cranial sources of S100B. *Br J Anaesth* 2001;86:601.
274. Kindblom LG, Lodding P, Rosengren L, Baudier J and Haglid K. S-100 protein in melanocytic tumors. An immunohistochemical investigation of benign and malignant melanocytic tumors and metastases of malignant melanoma and a characterization of the antigen in comparison to human brain. *Acta Pathol Microbiol Immunol Scand [A]* 1984;92:219-230.
275. Hidaka H, Endo T, Kawamoto S, Yamada E, Umekawa H, Tanabe K, *et al.* Purification and characterization of adipose tissue S-100b protein. *J Biol Chem* 1983;258:2705-2709.
276. Takahashi K, Isobe T, Ohtsuki Y, Sonobe H, Yamaguchi H and Akagi T. S-100 protein positive human T-lymphocyte. *Am J Clin Pathol* 1985;83:69-72.
277. Usui A, Kato K, Abe T, Murase M, Tanaka M and Takeuchi E. S-100a0 protein in blood and urine during open-heart surgery. *Clin Chem* 1989;35:1942-1944.
278. Cooper EH. Neuron-specific enolase. *Int J Biol Markers* 1994;9:205-210.

279. Marangos PJ, Schmechel D, Parma AM, Clark RL and Goodwin FK. Measurement of neuron-specific (NSE) and non-neuronal (NNE) isoenzymes of enolase in rat, monkey and human nervous tissue. *J Neurochem* 1979;33:319-329.
280. Kato K, Ishiguro Y, Suzuki F, Ito A and Semba R. Distribution of nervous system-specific forms of enolase in peripheral tissues. *Brain Res* 1982;237:441-448.
281. Tapia FJ, Polak JM, Barbosa AJ, Bloom SR, Marangos PJ, Dermody C, *et al.* Neuron-specific enolase is produced by neuroendocrine tumours. *Lancet* 1981;1:808-811.
282. Royds JA, Davies-Jones GA, Lewtas NA, Timperley WR and Taylor CB. Enolase isoenzymes in the cerebrospinal fluid of patients with diseases of the nervous system. *J Neurol Neurosurg Psychiatry* 1983;46:1031-1036.
283. Hay E, Royds JA, Davies-Jones GA, Lewtas NA, Timperley WR and Taylor CB. Cerebrospinal fluid enolase in stroke. *J Neurol Neurosurg Psychiatry* 1984;47:724-729.
284. Woertgen C and Rothoerl RD. Serum S-100B protein in severe head injury. *Neurosurgery* 2000;46:1026-1027.
285. Missler U, Wiesmann M, Friedrich C and Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. *Stroke* 1997;28:1956-1960.
286. Abraha HD, Butterworth RJ, Bath PM, Wassif WS, Garthwaite J and Sherwood RA. Serum S-100 protein, relationship to clinical outcome in acute stroke. *Ann Clin Biochem* 1997;34 (Pt 4):366-370.
287. Buttner T, Lack B, Jager M, Wunsche W, Kuhn W, Muller T, *et al.* Serum levels of neuron-specific enolase and s-100 protein after single tonic-clonic seizures. *J Neurol* 1999;246:459-461.
288. DeGiorgio CM, Correale JD, Gott PS, Ginsburg DL, Bracht KA, Smith T, *et al.* Serum neuron-specific enolase in human status epilepticus. *Neurology* 1995;45:1134-1137.
289. Rosen H, Sunnerhagen KS, Herlitz J, Blomstrand C and Rosengren L. Serum levels of the brain-derived proteins S-100 and NSE predict long-term outcome after cardiac arrest. *Resuscitation* 2001;49:183-191.
290. Ashraf S, Bhattacharya K, Tian Y and Watterson K. Cytokine and S100B levels in paediatric patients undergoing corrective cardiac surgery with or without total circulatory arrest. *Eur J Cardiothorac Surg* 1999;16:32-37.

291. Ingebrigtsen T, Romner B, Marup-Jensen S, Dons M, Lundqvist C, Bellner J, *et al.* The clinical value of serum S-100 protein measurements in minor head injury: a Scandinavian multicentre study. *Brain Inj* 2000;14:1047-1055.
292. Raabe A, Grolms C, Keller M, Dohnert J, Sorge O and Seifert V. Correlation of computed tomography findings and serum brain damage markers following severe head injury. *Acta Neurochir (Wien)* 1998;140:787-791; discussion 791-782.
293. Romner B, Ingebrigtsen T, Kongstad P and Borgesen SE. Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings. *J Neurotrauma* 2000;17:641-647.
294. Pleines UE, Morganti-Kossmann MC, Rancan M, Joller H, Trentz O and Kossmann T. S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. *J Neurotrauma* 2001;18:491-498.
295. Ciccarelli R, Di Iorio P, Bruno V, Battaglia G, D'Alimonte I, D'Onofrio M, *et al.* Activation of A(1) adenosine or mGlu3 metabotropic glutamate receptors enhances the release of nerve growth factor and S-100beta protein from cultured astrocytes. *Glia* 1999;27:275-281.
296. Azmitia EC and Whitaker-Azmitia PM. Awakening the sleeping giant: anatomy and plasticity of the brain serotonergic system. *J Clin Psychiatry* 1991;52 Suppl:4-16.
297. Whitaker-Azmitia PM and Azmitia EC. Astroglial 5-HT1a receptors and S-100 beta in development and plasticity. *Perspect Dev Neurobiol* 1994;2:233-238.
298. Whitaker-Azmitia PM, Murphy R and Azmitia EC. Stimulation of astroglial 5-HT1A receptors releases the serotonergic growth factor, protein S-100, and alters astroglial morphology. *Brain Res* 1990;528:155-158.
299. Suzuki F, Kato K, Kato T and Ogasawara N. S-100 protein in clonal astrogloma cells is released by adrenocorticotrophic hormone and corticotropin-like intermediate-lobe peptide. *J Neurochem* 1987;49:1557-1563.
300. Marshak DR. S100 beta as a neurotrophic factor. *Prog Brain Res* 1990;86:169-181.
301. Nilsson P, Hillered L, Ponten U and Ungerstedt U. Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. *J Cereb Blood Flow Metab* 1990;10:631-637.
302. Headrick JP, Bendall MR, Faden AI and Vink R. Dissociation of adenosine levels from bioenergetic state in experimental brain trauma: potential role in secondary injury. *J Cereb Blood Flow Metab* 1994;14:853-861.

303. Rudolphi KA, Schubert P, Parkinson FE and Fredholm BB. Adenosine and brain ischemia. *Cerebrovasc Brain Metab Rev* 1992;4:346-369.
304. Janeczko K. The proliferative response of S-100 protein-positive glial cells to injury in the neonatal rat brain. *Brain Res* 1991;564:86-90.
305. Janeczko K. Age-dependent changes in the proliferative response of S-100 protein-positive glial cells to injury in the rat brain. *Int J Dev Neurosci* 1994;12:431-440.
306. Miyake T, Hattori T, Fukuda M and Kitamura T. Reactions of S-100-positive glia after injury of mouse cerebral cortex. *Brain Res* 1989;489:31-40.
307. Tanaka H, Araki M and Masuzawa T. Reaction of astrocytes in the gerbil hippocampus following transient ischemia: immunohistochemical observations with antibodies against glial fibrillary acidic protein, glutamine synthetase, and S-100 protein. *Exp Neurol* 1992;116:264-274.
308. Schmidt-Kastner R, Szymas J and Hossmann KA. Immunohistochemical study of glial reaction and serum-protein extravasation in relation to neuronal damage in rat hippocampus after ischemia. *Neuroscience* 1990;38:527-540.
309. Raabe A, Grolms C and Seifert V. Serum markers of brain damage and outcome prediction in patients after severe head injury. *Br J Neurosurg* 1999;13:56-59.
310. Ross SA, Cunningham RT, Johnston CF and Rowlands BJ. Neuron-specific enolase as an aid to outcome prediction in head injury. *Br J Neurosurg* 1996;10:471-476.
311. Raabe A and Seifert V. Fatal secondary increase in serum S-100B protein after severe head injury. Report of three cases. *J Neurosurg* 1999;91:875-877.
312. Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Clark RS, Ruppel RA, *et al.* Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. *Pediatrics* 2002;109:E31.
313. Clark RS, Kochanek PM, Adelson PD, Bell MJ, Carcillo JA, Chen M, *et al.* Increases in bcl-2 protein in cerebrospinal fluid and evidence for programmed cell death in infants and children after severe traumatic brain injury. *J Pediatr* 2000;137:197-204.
314. Ruppel RA, Clark RS, Bayir H, Satchell MA and Kochanek PM. Critical mechanisms of secondary damage after inflicted head injury in infants and children. *Neurosurg Clin N Am* 2002;13:169-182, v.
315. Ruppel RA, Kochanek PM, Adelson PD, Rose ME, Wisniewski SR, Bell MJ, *et al.* Excitatory amino acid concentrations in ventricular cerebrospinal fluid after

severe traumatic brain injury in infants and children: the role of child abuse. *J Pediatr* 2001;138:18-25.

316. Jackson RG, Samra GS, Radcliffe J, Clark GH and Price CP. The early fall in levels of S-100 beta in traumatic brain injury. *Clin Chem Lab Med* 2000;38:1165-1167.

317. Raabe A, Grolms C, Sorge O, Zimmermann M and Seifert V. Serum S-100B protein in severe head injury. *Neurosurgery* 1999;45:477-483.

318. Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC and Dinarello CA. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 1990;75:40-47.

319. Maruo N, Morita I, Shirao M and Murota S. IL-6 increases endothelial permeability in vitro. *Endocrinology* 1992;131:710-714.

320. Woodroffe MN, Sarna GS, Wadhwa M, Hayes GM, Loughlin AJ, Tinker A, *et al.* Detection of interleukin-1 and interleukin-6 in adult rat brain, following mechanical injury, by in vivo microdialysis: evidence of a role for microglia in cytokine production. *J Neuroimmunol* 1991;33:227-236.

321. Yan HQ, Banos MA, Herregodts P, Hooghe R and Hooghe-Peters EL. Expression of interleukin (IL)-1 beta, IL-6 and their respective receptors in the normal rat brain and after injury. *Eur J Immunol* 1992;22:2963-2971.

322. Shohami E, Novikov M, Bass R, Yamin A and Gallily R. Closed head injury triggers early production of TNF alpha and IL-6 by brain tissue. *J Cereb Blood Flow Metab* 1994;14:615-619.

323. Taupin V, Toulmond S, Serrano A, Benavides J and Zavala F. Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion. Influence of pre- and post-traumatic treatment with Ro5 4864, a peripheral-type (p site) benzodiazepine ligand. *J Neuroimmunol* 1993;42:177-185.

324. Hariri RJ, Chang VA, Barie PS, Wang RS, Sharif SF and Ghajar JB. Traumatic injury induces interleukin-6 production by human astrocytes. *Brain Res* 1994;636:139-142.

325. Aloisi F, Care A, Borsellino G, Gallo P, Rosa S, Bassani A, *et al.* Production of hemolymphopoietic cytokines (IL-6, IL-8, colony-stimulating factors) by normal human astrocytes in response to IL-1 beta and tumor necrosis factor-alpha. *J Immunol* 1992;149:2358-2366.

326. Bazzoni F, Cassatella MA, Rossi F, Ceska M, Dewald B and Baggiolini M. Phagocytosing neutrophils produce and release high amounts of the neutrophil-activating peptide 1/interleukin 8. *J Exp Med* 1991;173:771-774.

327. Proost P, Wuyts A and van Damme J. The role of chemokines in inflammation. *Int J Clin Lab Res* 1996;26:211-223.
328. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. *J Infect Dis* 1991;163:1177-1184.
329. Uzan M, Tanriover N, Bozkus H, Gumustas K, Guzel O and Kuday C. Nitric oxide (NO) metabolism in the cerebrospinal fluid of patients with severe head injury. Inflammation as a possible cause of elevated no metabolites. *Surg Neurol* 2001;56:350-356.
330. O'Farrell AM, Liu Y, Moore KW and Mui AL. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. *Embo J* 1998;17:1006-1018.
331. Romano MF, Lamberti A, Petrella A, Bisogni R, Tassone PF, Formisano S, *et al.* IL-10 inhibits nuclear factor-kappa B/Rel nuclear activity in CD3-stimulated human peripheral T lymphocytes. *J Immunol* 1996;156:2119-2123.
332. Benveniste EN, Tang LP and Law RM. Differential regulation of astrocyte TNF-alpha expression by the cytokines TGF-beta, IL-6 and IL-10. *Int J Dev Neurosci* 1995;13:341-349.
333. Bogdan C, Paik J, Vodovotz Y and Nathan C. Contrasting mechanisms for suppression of macrophage cytokine release by transforming growth factor-beta and interleukin-10. *J Biol Chem* 1992;267:23301-23308.
334. Chao CC, Hu S, Sheng WS and Peterson PK. Tumor necrosis factor-alpha production by human fetal microglial cells: regulation by other cytokines. *Dev Neurosci* 1995;17:97-105.
335. Cassatella MA, Meda L, Gasperini S, Calzetti F and Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. *J Exp Med* 1994;179:1695-1699.
336. Joyce DA, Gibbons DP, Green P, Steer JH, Feldmann M and Brennan FM. Two inhibitors of pro-inflammatory cytokine release, interleukin-10 and interleukin-4, have contrasting effects on release of soluble p75 tumor necrosis factor receptor by cultured monocytes. *Eur J Immunol* 1994;24:2699-2705.
337. Balasingam V and Yong VW. Attenuation of astroglial reactivity by interleukin-10. *J Neurosci* 1996;16:2945-2955.
338. Lodge PA and Sriram S. Regulation of microglial activation by TGF-beta, IL-10, and CSF-1. *J Leukoc Biol* 1996;60:502-508.

339. Bogdan C and Nathan C. Modulation of macrophage function by transforming growth factor beta, interleukin-4, and interleukin-10. *Ann N Y Acad Sci* 1993;685:713-739.
340. Shimonkevitz R, Bar-Or D, Harris L, Dole K, McLaughlin L and Yukl R. Transient monocyte release of interleukin-10 in response to traumatic brain injury. *Shock* 1999;12:10-16.
341. Spertini O, Luscinskas FW, Kansas GS, Munro JM, Griffin JD, Gimbrone MA, Jr., *et al.* Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. *J Immunol* 1991;147:2565-2573.
342. Schleiffenbaum B, Spertini O and Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. *J Cell Biol* 1992;119:229-238.
343. Suzuki H, Sato S, Suzuki Y, Oka M, Tsuchiya T, Iino I, *et al.* Endothelin immunoreactivity in cerebrospinal fluid of patients with subarachnoid haemorrhage. *Ann Med* 1990;22:233-236.
344. Kraus GE, Bucholz RD, Yoon KW, Knuepfer MM and Smith KR, Jr. Cerebrospinal fluid endothelin-1 and endothelin-3 levels in normal and neurosurgical patients: a clinical study and literature review. *Surg Neurol* 1991;35:20-29.
345. Yang Y, Huang W and Lu X. Early changes of endothelin, nitric oxide and arginine-vasopressin in patients with acute cerebral injury. *Chin J Traumatol* 2002;5:259-262.
346. Takahashi H, Sato H and Tsuji Y. Biochemical markers in the acute stage of head injury--aldosterone and CK-BB. *Neurol Med Chir (Tokyo)* 1989;29:192-195.
347. Pleines UE, Stover JF, Kossmann T, Trentz O and Morganti-Kossmann MC. Soluble ICAM-1 in CSF coincides with the extent of cerebral damage in patients with severe traumatic brain injury. *J Neurotrauma* 1998;15:399-409.
348. Fridriksson T, Kini N, Walsh-Kelly C and Hennes H. Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: a pilot study. *Acad Emerg Med* 2000;7:816-820.
349. Zweig MH and Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-577.
350. Gebhard F, Pfetsch H, Steinbach G, Strecker W, Kinzl L and Bruckner UB. Is interleukin 6 an early marker of injury severity following major trauma in humans? *Arch Surg* 2000;135:291-295.

351. Woiciechowsky C, Schoning B, Cobanov J, Lanksch WR, Volk HD and Docke WD. Early IL-6 plasma concentrations correlate with severity of brain injury and pneumonia in brain-injured patients. *J Trauma* 2002;52:339-345.
352. Minambres E, Cemborain A, Sanchez-Velasco P, Gandarillas M, Diaz-Reganon G, Sanchez-Gonzalez U, *et al.* Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. *Crit Care Med* 2003;31:933-938.
353. Piazza O, Storti MP, Cotena S, Stoppa F, Perrotta D, Esposito G, *et al.* S100B is not a reliable prognostic index in paediatric TBI. *Pediatr Neurosurg* 2007;43:258-264.
354. Vos PE, Lamers KJ, Hendriks JC, van Haaren M, Beems T, Zimmerman C, *et al.* Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology* 2004;62:1303-1310.
355. Chiaretti A, Antonelli A, Riccardi R, Genovese O, Pezzotti P, Di Rocco C, *et al.* Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children. *Eur J Paediatr Neurol* 2007.
356. Chiaretti A, Genovese O, Aloe L, Antonelli A, Piastra M, Polidori G, *et al.* Interleukin 1beta and interleukin 6 relationship with paediatric head trauma severity and outcome. *Childs Nerv Syst* 2005;21:185-193; discussion 194.
357. Piazza O, Russo E, Cotena S, Esposito G and Tufano R. Elevated S100B levels do not correlate with the severity of encephalopathy during sepsis. *Br J Anaesth* 2007;99:518-521.
358. Spinella PC, Dominguez T, Drott HR, Huh J, McCormick L, Rajendra A, *et al.* S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. *Crit Care Med* 2003;31:939-945.
359. Bandyopadhyay S, Hennes H, Gorelick MH, Wells RG and Walsh-Kelly CM. Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. *Acad Emerg Med* 2005;12:732-738.
360. Arand M, Melzner H, Kinzl L, Bruckner UB and Gebhard F. Early inflammatory mediator response following isolated traumatic brain injury and other major trauma in humans. *Langenbecks Arch Surg* 2001;386:241-248.
361. Shultz EK. Multivariate receiver-operating characteristic curve analysis: prostate cancer screening as an example. *Clin Chem* 1995;41:1248-1255.
362. Lindenberg R and Freytag E. Morphology of brain lesions from blunt trauma in early infancy. *Arch Pathol* 1969;87:298-305.

363. Freytag E. Autopsy findings in head injuries from blunt forces. Statistical evaluation of 1,367 cases. *Arch Pathol* 1963;75:402-413.
364. Maloney AF and Whatmore WJ. Clinical and pathological observations in fatal head injuries. A 5-year survey of 173 cases. *Br J Surg* 1969;56:23-31.
365. Strich SJ. The pathology of brain damage due to blunt head injuries. In: Walker A. E., Caveness W. F. and Critchley M., editors. The late effects of head injury: Springfield; 1969. p. 501-524.
366. Lindenberg R. Trauma of meninges and brain. In: Minckler J., editor. Pathology of the nervous system. New York: McGraw-Hill; 1971. p. 1705-1765.
367. Hardman JM. The pathology of traumatic brain injuries. In: Thompson R. A. and Green J. R., editors. Advances in Neurology. New York: Raven Press; 1979. p. 15-50.
368. Sevtitt S. Fatal road accidents in Birmingham: times to death and their causes. *Injury* 1973;4:281-293.
369. Adams JH, Graham DI, Scott G, Parker LS and Doyle D. Brain damage in fatal non-missile head injury. *J Clin Pathol* 1980;33:1132-1145.
370. Bruce DA, Alavi A, Bilaniuk L, Dolinskas C, Obrist W and Uzzell B. Diffuse cerebral swelling following head injuries in children: the syndrome of "malignant brain edema". *J Neurosurg* 1981;54:170-178.
371. Blumbergs PC, Scott G, Manavis J, Wainwright H, Simpson DA and McLean AJ. Staining of amyloid precursor protein to study axonal damage in mild head injury. *Lancet* 1994;344:1055-1056.
372. Gentleman SM, Nash MJ, Sweeting CJ, Graham DI and Roberts GW. Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neurosci Lett* 1993;160:139-144.
373. Gentleman SM, Roberts GW, Gennarelli TA, Maxwell WL, Adams JH, Kerr S, *et al.* Axonal injury: a universal consequence of fatal closed head injury? *Acta Neuropathol (Berl)* 1995;89:537-543.
374. Hortobagyi T, Wise S, Hunt N, Cary N, Djurovic V, Fegan-Earl A, *et al.* Traumatic axonal damage in the brain can be detected using beta-APP immunohistochemistry within 35 min after head injury to human adults. *Neuropathol Appl Neurobiol* 2007;33:226-237.
375. Kaur B, Rutty GN and Timperley WR. The possible role of hypoxia in the formation of axonal bulbs. *J Clin Pathol* 1999;52:203-209.

376. Dolinak D, Smith C and Graham DI. Hypoglycaemia is a cause of axonal injury. *Neuropathol Appl Neurobiol* 2000;26:448-453.
377. Becher JC, Bell JE, Keeling JW, McIntosh N and Wyatt B. The Scottish perinatal neuropathology study: clinicopathological correlation in early neonatal deaths. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F399-407.
378. The Royal Liverpool Children's Hospital Inquiry Report (Alder Hey). London: The Stationery Office; 2001.
379. Independent Review Group on Retention of Organs at Post-mortem; 2001.
380. Human tissue and biological samples for use in research; operational and ethical guidelines. London: Medical Research Council; 2001.
381. Adams JH, Doyle D, Ford I, Gennarelli TA, Graham DI and McLellan DR. Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* 1989;15:49-59.
382. Peerless SJ and Rewcastle NB. Shear injuries of the brain. *Can Med Assoc J* 1967;96:577-582.
383. Strich SJ. Diffuse degeneration of the cerebral white matter in severe dementia following head injury. *J Neurol Neurosurg Psychiatry* 1956;19:163-185.
384. Strich SJ. Shearing of nerve fibres as a cause of brain damage due to head injury: A pathological study of twenty cases. *Lancet* 1961;278:443-448.
385. Zimmerman RA, Bilaniuk LT and Genneralli T. Computed tomography of shearing injuries of the cerebral white matter. *Radiology* 1978;127:393-396.
386. Holbourn AHS. Mechanisms of head injuries. *Lancet* 1943;242:438-441.
387. Holbourn AHS. The mechanics of brain injuries. *British Medical Bulletin* 1945;3:147-149.
388. Ommaya AK and Gennarelli TA. Cerebral concussion and traumatic unconsciousness. Correlation of experimental and clinical observations of blunt head injuries. *Brain* 1974;97:633-654.
389. Jaspan T, Narborough G, Punt JA and Lowe J. Cerebral contusional tears as a marker of child abuse--detection by cranial sonography. *Pediatr Radiol* 1992;22:237-245.
390. Crompton R. Closed head injury in children. In: Crompton R., editor. Closed head injury in children. London: Edward Arnold; 1985.

391. Adams JH, Graham DI, Murray LS and Scott G. Diffuse axonal injury due to nonmissile head injury in humans: an analysis of 45 cases. *Ann Neurol* 1982;12:557-563.
392. Blumbergs PC, Jones NR and North JB. Diffuse axonal injury in head trauma. *J Neurol Neurosurg Psychiatry* 1989;52:838-841.
393. Wilkinson AE, Bridges LR and Sivaloganathan S. Correlation of survival time with size of axonal swellings in diffuse axonal injury. *Acta Neuropathol (Berl)* 1999;98:197-202.
394. Leclercq PD, Stephenson MS, Murray LS, McIntosh TK, Graham DI and Gentleman SM. Simple morphometry of axonal swellings cannot be used in isolation for dating lesions after traumatic brain injury. *J Neurotrauma* 2002;19:1183-1192.
395. Gorrie C, Oakes S, Duflou J, Blumbergs P and Waite PM. Axonal injury in children after motor vehicle crashes: extent, distribution, and size of axonal swellings using beta-APP immunohistochemistry. *J Neurotrauma* 2002;19:1171-1182.
396. Denny-Brown D and Ritchie Russell W. Experimental cerebral contusion. *Brain* 1941;64:93-164.
397. Gennarelli TA, Adams JH and Graham DI. Acceleration induced head injury in the monkey. I. The model, its mechanical and physiological correlates. *Acta Neuropathol Suppl (Berl)* 1981;7:23-25.
398. Gennarelli TA, Thibault LE, Adams JH, Graham DI, Thompson CJ and Marcincin RP. Diffuse axonal injury and traumatic coma in the primate. *Ann Neurol* 1982;12:564-574.
399. Jellinger K and Seitelberger F. Protracted post-traumatic encephalopathy. Pathology, pathogenesis and clinical implications. *J Neurol Sci* 1970;10:51-94.
400. Oehmichen M, Meissner C, Schmidt V, Pedal I and König HG. Pontine axonal injury after brain trauma and nontraumatic hypoxic- ischemic brain damage. *Int J Legal Med* 1999;112:261-267.
401. Niess C, Grauel U, Toennes SW and Bratzke H. Incidence of axonal injury in human brain tissue. *Acta Neuropathol* 2002;104:79-84.
402. Smith C, Graham DI, Geddes JF and Whitwell HL. The interpretation of beta-APP immunoreactivity: a response to C. Neiss et al., *Acta Neuropathol* (2002) 104:79. *Acta Neuropathol* 2003;106:97-98.
403. Diaz-Arrastia R and Baxter VK. Genetic factors in outcome after traumatic brain injury: what the human genome project can teach us about brain trauma. *J Head Trauma Rehabil* 2006;21:361-374.

404. Saupe J, Shearer MJ and Kohlmeier M. Phylloquinone transport and its influence on gamma-carboxyglutamate residues of osteocalcin in patients on maintenance hemodialysis. *Am J Clin Nutr* 1993;58:204-208.
405. Giraud V, Naveau S, Betoulle D, Abella A, Bardou M, Borotto E, *et al.* [Influence of apolipoprotein E polymorphism in alcoholic cirrhosis]. *Gastroenterol Clin Biol* 1998;22:571-575.
406. Weir CJ, McCarron MO, Muir KW, Dyker AG, Bone I, Lees KR, *et al.* Apolipoprotein E genotype, coagulation, and survival following acute stroke. *Neurology* 2001;57:1097-1100.
407. Olson JD, Kaufman HH, Moake J, O'Gorman TW, Hoots K, Wagner K, *et al.* The incidence and significance of hemostatic abnormalities in patients with head injuries. *Neurosurgery* 1989;24:825-832.
408. Piek J, Chesnut RM, Marshall LF, van Berkum-Clark M, Klauber MR, Blunt BA, *et al.* Extracranial complications of severe head injury. *J Neurosurg* 1992;77:901-907.
409. Pondaag W. Disseminated intravascular coagulation related to outcome in head injury. *Acta Neurochir Suppl (Wien)* 1979;28:98-102.
410. Liaquat I, Dunn LT, Nicoll JA, Teasdale GM and Norrie JD. Effect of apolipoprotein E genotype on hematoma volume after trauma. *J Neurosurg* 2002;96:90-96.
411. Gando S, Nanzaki S and Kemmotsu O. Coagulofibrinolytic changes after isolated head injury are not different from those in trauma patients without head injury. *J Trauma* 1999;46:1070-1076; discussion 1076-1077.
412. Hoots WK. Coagulation disorders in head-injured patients. In: Narayan R. K., Wilberger J. E. and Povlishock J. T., editors. *Neurotrauma*. New York: McGraw-Hill; 1996. p. 673-688.
413. Lu D, Mahmood A, Goussev A, Qu C, Zhang ZG and Chopp M. Delayed thrombosis after traumatic brain injury in rats. *J Neurotrauma* 2004;21:1756-1766.
414. Lu D, Mahmood A, Goussev A, Schallert T, Qu C, Zhang ZG, *et al.* Atorvastatin reduction of intravascular thrombosis, increase in cerebral microvascular patency and integrity, and enhancement of spatial learning in rats subjected to traumatic brain injury. *J Neurosurg* 2004;101:813-821.
415. Stein SC, Graham DI, Chen XH and Smith DH. Association between intravascular microthrombosis and cerebral ischemia in traumatic brain injury. *Neurosurgery* 2004;54:687-691; discussion 691.

416. Stein SC, Graham DI, Chen XH, Dunn L and Smith DH. Apo E genotype not associated with intravascular coagulation in traumatic brain injury. *Neurosci Lett* 2005;387:28-31.
417. Lynch JR, Pineda JA, Morgan D, Zhang L, Warner DS, Benveniste H, *et al.* Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Ann Neurol* 2002;51:113-117.
418. Quinn TJ, Smith C, Murray L, Stewart J, Nicoll JA and Graham DI. There is no evidence of an association in children and teenagers between the apolipoprotein E epsilon4 allele and post-traumatic brain swelling. *Neuropathol Appl Neurobiol* 2004;30:569-575.
419. McCarron MO, Hoffmann KL, DeLong DM, Gray L, Saunders AM and Alberts MJ. Intracerebral hemorrhage outcome: apolipoprotein E genotype, hematoma, and edema volumes. *Neurology* 1999;53:2176-2179.
420. Nicoll JA, Roberts GW and Graham DI. Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med* 1995;1:135-137.
421. Horsburgh K, Cole GM, Yang F, Savage MJ, Greenberg BD, Gentleman SM, *et al.* beta-amyloid (Abeta)42(43), abeta42, abeta40 and apoE immunostaining of plaques in fatal head injury. *Neuropathol Appl Neurobiol* 2000;26:124-132.
422. Macfarlane DP, Nicoll JA, Smith C and Graham DI. APOE epsilon4 allele and amyloid beta-protein deposition in long term survivors of head injury. *Neuroreport* 1999;10:3945-3948.
423. Han SH, Einstein G, Weisgraber KH, Strittmatter WJ, Saunders AM, Pericak-Vance M, *et al.* Apolipoprotein E is localized to the cytoplasm of human cortical neurons: a light and electron microscopic study. *J Neuropathol Exp Neurol* 1994;53:535-544.
424. Fleming LM, Weisgraber KH, Strittmatter WJ, Troncoso JC and Johnson GV. Differential binding of apolipoprotein E isoforms to tau and other cytoskeletal proteins. *Exp Neurol* 1996;138:252-260.
425. Strittmatter WJ, Saunders AM, Goedert M, Weisgraber KH, Dong LM, Jakes R, *et al.* Isoform-specific interactions of apolipoprotein E with microtubule-associated protein tau: implications for Alzheimer disease. *Proc Natl Acad Sci U S A* 1994;91:11183-11186.
426. Huang DY, Goedert M, Jakes R, Weisgraber KH, Garner CC, Saunders AM, *et al.* Isoform-specific interactions of apolipoprotein E with the microtubule-associated protein MAP2c: implications for Alzheimer's disease. *Neurosci Lett* 1994;182:55-58.

427. Lee VM, Balin BJ, Otvos L, Jr. and Trojanowski JQ. A68: a major subunit of paired helical filaments and derivatized forms of normal Tau. *Science* 1991;251:675-678.
428. Goedert M, Jakes R, Crowther RA, Six J, Lubke U, Vandermeeren M, *et al.* The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. *Proc Natl Acad Sci U S A* 1993;90:5066-5070.
429. Hayek T, Oiknine J, Brook JG and Aviram M. Increased plasma and lipoprotein lipid peroxidation in apo E-deficient mice. *Biochem Biophys Res Commun* 1994;201:1567-1574.
430. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D and Witztum JL. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb* 1994;14:605-616.
431. Miyata M and Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* 1996;14:55-61.
432. Holtzman DM, Pitas RE, Kilbridge J, Nathan B, Mahley RW, Bu G, *et al.* Low density lipoprotein receptor-related protein mediates apolipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. *Proc Natl Acad Sci U S A* 1995;92:9480-9484.
433. Gutman CR, Strittmatter WJ, Weisgraber KH and Matthew WD. Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. *J Neurosci* 1997;17:6114-6121.
434. Huang DY, Weisgraber KH, Strittmatter WJ and Matthew WD. Interaction of apolipoprotein E with laminin increases neuronal adhesion and alters neurite morphology. *Exp Neurol* 1995;136:251-257.
435. Macy M, Okano Y, Cardin AD, Avila EM and Harmony JA. Suppression of lymphocyte activation by plasma lipoproteins. *Cancer Res* 1983;43:2496s-2502s.
436. Pepe MG and Curtiss LK. Apolipoprotein E is a biologically active constituent of the normal immunoregulatory lipoprotein, LDL-In. *J Immunol* 1986;136:3716-3723.
437. Laskowitz DT, Goel S, Bennett ER and Matthew WD. Apolipoprotein E suppresses glial cell secretion of TNF alpha. *J Neuroimmunol* 1997;76:70-74.

438. Leira R, Rodriguez-Yanez M, Castellanos M, Blanco M, Nombela F, Sobrino T, *et al.* Hyperthermia is a surrogate marker of inflammation-mediated cause of brain damage in acute ischaemic stroke. *J Intern Med* 2006;260:343-349.
439. Wang JY, Zhou DH, Li J, Zhang M, Deng J, Gao C, *et al.* Association of soluble intercellular adhesion molecule 1 with neurological deterioration of ischemic stroke: The Chongqing Stroke Study. *Cerebrovasc Dis* 2006;21:67-73.
440. Blum A, Khazim K, Merei M, Peleg A, Blum N and Vaispapir V. The stroke trial - can we predict clinical outcome of patients with ischemic stroke by measuring soluble cell adhesion molecules (CAM)? *Eur Cytokine Netw* 2006;17:295-298.
441. Hernandez LA, Grisham MB, Twohig B, Arfors KE, Harlan JM and Granger DN. Role of neutrophils in ischemia-reperfusion-induced microvascular injury. *Am J Physiol* 1987;253:H699-703.
442. Zhang RL, Chopp M, Chen H and Garcia JH. Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *J Neurol Sci* 1994;125:3-10.
443. Gillick v West Norfolk and Wisbech Area Health Authority. *3 All ER* 1985;402.
444. McIntosh N, Bates P, Brykczynska G, Dunstan G, Goldman A, Harvey D, *et al.* Guidelines for the ethical conduct of medical research involving children. Royal College of Paediatrics, Child Health: Ethics Advisory Committee. *Arch Dis Child* 2000;82:177-182.
445. Biros MH, Lewis RJ, Olson CM, Runge JW, Cummins RO and Fost N. Informed consent in emergency research. Consensus statement from the Coalition Conference of Acute Resuscitation and Critical Care Researchers. *Jama* 1995;273:1283-1287.
446. Stenson BJ, Becher JC and McIntosh N. Neonatal research: the parental perspective. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F321-323.
447. Underwood JCE. Autopsies and clinical audit. In: Burton J. L. and Rutty G. N., editors. The hospital autopsy. London: Arnold; 2001. p. 170 - 177.
448. The Human Tissue Act. In; 1961.
449. Guidelines for the Retention of Tissues and Organs at Post-mortem Examination. London: Royal College of Pathologists; 2000.
450. Start RD, McCulloch TA, Benbow EW, Lauder I and Underwood JC. Clinical necropsy rates during the 1980s: the continued decline. *J Pathol* 1993;171:63-66.

451. Burton JL. The history of autopsy. In: Burton J. L. and Ruttly G. N., editors. The hospital autopsy. London: Arnold; 2001. p. 1 - 6.
452. Kmietowicz Z. Shortage of academics threatens rise in number of doctors. *BMJ* 2001;322:1442.
453. Quirke P. Diary of the manpower director: news from the front line. *Royal College of Pathology Bulletin* 2000;16 - 19.
454. Cox P. Perinatal pathology - is there a future after Alder Hey? *AcpNews* 2001;Spring:14 - 15.
455. Evans M-J. Leaving pathology: a personal view. *AcpNews* 2001;Spring:11 - 13.
456. Burton JL and Wells M. The Alder Hey affair: implications for pathology practice. *J Clin Pathol* 2001;54:820-823.
457. Underwood JC, Cotton DW and Stephenson TJ. Audit and necropsy. *Lancet* 1989;1:442.
458. Post Mortem Examination: considerations for best practice in the future. In: The future of Paediatric Pathology Service: Royal College of Paediatrics and Child Health; 2002. p. 21-25.
459. Adams JH, Jennett B, McLellan DR, Murray LS and Graham DI. The neuropathology of the vegetative state after head injury. *J Clin Pathol* 1999;52:804-806.
460. Adams JH, Graham DI and Jennett B. The structural basis of moderate disability after traumatic brain damage. *J Neurol Neurosurg Psychiatry* 2001;71:521-524.
461. Fitzpatrick MO, Dewar D, Teasdale GM and Graham DI. The neuronal cytoskeleton in acute brain injury. *Br J Neurosurg* 1998;12:313-317.
462. Dewar D, Underhill SM and Goldberg MP. Oligodendrocytes and ischemic brain injury. *J Cereb Blood Flow Metab* 2003;23:263-274.
463. Gillard JH, Papadakis NG, Martin K, Price CJ, Warburton EA, Antoun NM, *et al.* MR diffusion tensor imaging of white matter tract disruption in stroke at 3 T. *Br J Radiol* 2001;74:642-647.
464. Pantoni L, Leys D, Fazekas F, Longstreth WT, Jr., Inzitari D, Wallin A, *et al.* Role of white matter lesions in cognitive impairment of vascular origin. *Alzheimer Dis Assoc Disord* 1999;13 Suppl 3:S49-54.

465. Di Stefano G, Bachevalier J, Levin HS, Song JX, Scheibel RS and Fletcher JM. Volume of focal brain lesions and hippocampal formation in relation to memory function after closed head injury in children. *J Neurol Neurosurg Psychiatry* 2000;69:210-216.
466. Benavidez DA, Fletcher JM, Hannay HJ, Bland ST, Caudle SE, Mendelsohn DB, *et al.* Corpus callosum damage and interhemispheric transfer of information following closed head injury in children. *Cortex* 1999;35:315-336.
467. Teasdale GM, Murray GD and Nicoll JA. The association between APOE epsilon4, age and outcome after head injury: a prospective cohort study. *Brain* 2005;128:2556-2561.
468. White F, Nicoll JA, Roses AD and Horsburgh K. Impaired neuronal plasticity in transgenic mice expressing human apolipoprotein E4 compared to E3 in a model of entorhinal cortex lesion. *Neurobiol Dis* 2001;8:611-625.
469. Haqqani AS, Hutchison JS, Ward R and Stanimirovic DB. Biomarkers and diagnosis; protein biomarkers in serum of pediatric patients with severe traumatic brain injury identified by ICAT-LC-MS/MS. *J Neurotrauma* 2007;24:54-74.
470. Dewar D, Yam P and McCulloch J. Drug development for stroke: importance of protecting cerebral white matter. *Eur J Pharmacol* 1999;375:41-50.
471. Feigin VL, Anderson CS, Rodgers A, Anderson NE and Gunn AJ. The emerging role of induced hypothermia in the management of acute stroke. *J Clin Neurosci* 2002;9:502-507.
472. Koizumi H and Povlishock JT. Posttraumatic hypothermia in the treatment of axonal damage in an animal model of traumatic axonal injury. *J Neurosurg* 1998;89:303-309.
473. Maxwell WL, Donnelly S, Sun X, Fenton T, Puri N and Graham DI. Axonal cytoskeletal responses to nondisruptive axonal injury and the short-term effects of posttraumatic hypothermia. *J Neurotrauma* 1999;16:1225-1234.
474. Maxwell WL, Islam MN, Graham DI and Gennarelli TA. A qualitative and quantitative analysis of the response of the retinal ganglion cell soma after stretch injury to the adult guinea-pig optic nerve. *J Neurocytol* 1994;23:379-392.
475. Zhao H, Steinberg GK and Sapolsky RM. General versus specific actions of mild-moderate hypothermia in attenuating cerebral ischemic damage. *J Cereb Blood Flow Metab* 2007;27:1879-1894.
476. Berger R, Jensen A, Hossmann KA and Paschen W. Effect of mild hypothermia during and after transient in vitro ischemia on metabolic disturbances in hippocampal slices at different stages of development. *Brain Res Dev Brain Res* 1998;105:67-77.

477. Aoki M, Tamatani M, Taniguchi M, Yamaguchi A, Bando Y, Kasai K, *et al.* Hypothermic treatment restores glucose regulated protein 78 (GRP78) expression in ischemic brain. *Brain Res Mol Brain Res* 2001;95:117-128.
478. Macleod MR, Johansson IM, Soderstrom I, Lai M, Gido G, Wieloch T, *et al.* Mineralocorticoid receptor expression and increased survival following neuronal injury. *Eur J Neurosci* 2003;17:1549-1555.
479. Mouritzen CV and Andersen MN. Mechanisms of ventricular fibrillation during hypothermia. Relative changes in myocardial refractory period and conduction velocity. *J Thorac Cardiovasc Surg* 1966;51:579-584.
480. Rohrer MJ and Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med* 1992;20:1402-1405.
481. Bohn DJ, Biggar WD, Smith CR, Conn AW and Barker GA. Influence of hypothermia, barbiturate therapy, and intracranial pressure monitoring on morbidity and mortality after near-drowning. *Crit Care Med* 1986;14:529-534.
482. Biggart MJ and Bohn DJ. Effect of hypothermia and cardiac arrest on outcome of near-drowning accidents in children. *J Pediatr* 1990;117:179-183.
483. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, *et al.* Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002;346:557-563.
484. Levin AV. Ophthalmology of shaken baby syndrome. *Neurosurg Clin N Am* 2002;13:201-211, vi.
485. Hachimi-Idrissi S, Corne L, Ebinger G, Michotte Y and Huyghens L. Mild hypothermia induced by a helmet device: a clinical feasibility study. *Resuscitation* 2001;51:275-281.
486. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, *et al.* Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 2005;365:663-670.
487. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, *et al.* Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 2005;353:1574-1584.
488. Leonov Y, Sterz F, Safar P and Radovsky A. Moderate hypothermia after cardiac arrest of 17 minutes in dogs. Effect on cerebral and cardiac outcome. *Stroke* 1990;21:1600-1606.

489. Clifton GL, Jiang JY, Lyeth BG, Jenkins LW, Hamm RJ and Hayes RL. Marked protection by moderate hypothermia after experimental traumatic brain injury. *J Cereb Blood Flow Metab* 1991;11:114-121.
490. Dietrich WD, Alonso O, Busto R, Globus MY and Ginsberg MD. Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. *Acta Neuropathol (Berl)* 1994;87:250-258.
491. Clark RS, Kochanek PM, Marion DW, Schiding JK, White M, Palmer AM, *et al.* Mild posttraumatic hypothermia reduces mortality after severe controlled cortical impact in rats. *J Cereb Blood Flow Metab* 1996;16:253-261.
492. Tisherman SA, Rodriguez A and Safar P. Therapeutic hypothermia in traumatology. *Surg Clin North Am* 1999;79:1269-1289.
493. Sambasivan M. Hypothermia in craniocerebral trauma revisited. *Neurol India* 1999;47:255-258.
494. Tasker RC, Morris KP, Forsyth RJ, Hawley CA and Parslow RC. Severe head injury in children: emergency access to neurosurgery in the United Kingdom. *Emerg Med J* 2006;23:519-522.

LIST OF ABBREVIATIONS

A&E	Accident and Emergency Department
APO E	Apolipoprotein E gene
apo E	Apolipoprotein E (Gene product of the Apolipoprotein E gene)
AIS	Abbreviated Injury Scale
ATP	Adenosine triphosphate
AUC	Area under the curve
BBB	Blood-brain barrier
CDC	Computerized Data Collection
CMRO ₂	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CPP	Cerebral perfusion pressure
GCS	Glasgow Coma Scale
GOS	Glasgow Outcome Scale score
ICP	Intracranial pressure
ICU	Intensive Care Unit
IL-1 β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
ISS	Injury Severity Score
MAP	Mean arterial blood pressure
MultiROC	Multivariate Receiver Operator Characteristic curve
Na ⁺	Sodium
NAHI	Non-accidental head injury
NGH	Newcastle General Hospital
NHS	National Health Service
PCR	Polymerase chain reaction
PICU	Paediatric Intensive Care Unit
PTI	Cumulative pressure-time index
PTIc	Cumulative pressure-time index for cerebral perfusion pressure
PTIi	Cumulative pressure-time index for intracranial pressure
PTS	Paediatric Trauma Score
RHSC	Royal Hospital for Sick Children, Edinburgh
ROC	Receiver Operator Characteristic curve
RTA	Road traffic accident
RTS	Revised Trauma Score
SICAM	Soluble Intracellular Adhesion Molecule
SjO ₂	Global brain oxygen extraction
TBI	Traumatic brain injury
TCDB	Traumatic Coma Data Bank
TNF- α	tumour necrosis factor alpha
TRISS	Trauma Score-Injury Severity Score
WGH	Western General Hospital, Edinburgh

ACKNOWLEDGEMENTS

I would like to send my special thanks to Prof. R. A. Minns (my Principal Supervisor), Mrs. P. A. Jones ('Auntie Pat' and my unofficial supervisor), Prof. J Bell and Dr. C Smith (my two other supervisors) for all their guidance, help, and support throughout the course of this work. Without them, this thesis would not have been possible.

I am forever grateful to our Newcastle collaborators especially Dr. I. R. Chambers, Prof. A. D. Mendelow, Dr. R. Forsyth, Dr. B. Fenton, and our local A&E, ICU and neurosurgical colleagues especially Dr. D. Simpson, Dr. J. Freeman, Dr. T. F. Beattie, Prof. P. J. D. Andrews, Mr. A. J. W. Steers, and Miss L. Myles for their support and help with the project. Without their collaborations, this project would not have been half as successful as it is. I would like to thank Dr. Magnus Hedberg for providing us with the complimentary use of the ICUPilot software. Without his generosity, data collection would have been delayed significantly at the Edinburgh RHSC site.

I am indebted to all the ICU staff in Edinburgh and Newcastle for their support and help in patient recruitments, Mrs. Rhona Stephen and Mr. Ian Croy for their invaluable advice in laboratory methodologies.

I would like to thank Prof. David Harrison for introducing me to the world of research and academia when I was only a medical student. Without his guidance, I would not have discovered my destined career path.

Finally, I would like to thank my parents and 'baby' sister for their support, encouragement, and patience during the course of this work (especially during stressful periods when I was almost impossible to live with). Without them, life would not have any meanings.

APPENDICES

APPENDIX I: AGE INDEX FOR PHYSIOLOGICAL DERANGEMENTS

Term	2 wks	yrs																		
		4	6	1 yr	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Raised ICP (mmHg)	4																			
MAP –low (mmHg)	40	38	39	44	45	50	46	45	49	50	51	53	54	56	60	57	58	58		
MAP-high (mmHg)	53	58	67	65	67	73	67	65	73	74	76	78	79	80	84	83	84	85		
BPsys-high (mmHg)	91	106	119	125	119	117	119	117	116	117	119	119	122	124	131	133	136	138		
CPP (mmHg)	40	38	39	44	45	50	46	45	49	50	51	53	54	56	60	57	58	58		
Hypoxia (Oxygen saturation %)	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90	90		
Pyrexia	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38		
Tachycardia	145	145	145	175	170	170	140	140	140	140	120	120	120	110	100	100	100	115		
Bradycardia	95	95	95	110	105	105	80	80	80	80	70	70	70	60	60	60	60	65		

Sources:

1. Systolic and Diastolic Blood Pressure. Second Task Force on Blood Pressure control. USA 1987; 72,000 subjects. Range used was 2 standard deviations from value given (*Pediatrics*, 1987; 79)
2. Mean Arterial Pressure (MAP). Calculated from above tables, using formula $MAP=(D+(S-D/3))$, where D= diastolic, and S = systolic
3. Intracranial Pressure (ICP). Minns, tabulated normal pressures described by earlier workers (*IN: Problems of Intracranial Pressure in childhood. London: MacKeith Press, 1991*)
4. Cerebral Perfusion Pressure (CPP). Little available information specific to children. Lowest normal limit of CPP in head trauma patients is usually considered to be the same as the lowest acceptable limit for MAP.
5. Heart Rate. Data as published by Shann (*IN: Drug Doses. Collective Pty Ltd., 1996*)
6. Hypoxia. Oxygen saturation, measured by a pulse oximeter, was considered outside normal values if it was $\leq 90\%$
7. Pyrexia. When Core temperature rises to 38°C

Appendix II

Neurochemical Marker Assay Procedures

Nexus Dx™ NSE Test Kit (Synx Pharma Inc.)

Reagent Supplied

- (1) 96 microtitration wells (arranged as twelve 8-well strips held in a strip holder) coated with monoclonal anti-NSE antibody
- (2) Five vials of Neuron Specific Enolase standards (0, 6.25, 25, 50, and 100 ng/ml)
- (3) One vial of Incubation Buffer
- (4) Three vials of NSE protein controls (low, medium and high level)
- (5) One vial of monoclonal anti-NSE immunoglobulin labelled as Horseradish Peroxidase (HRP) – Antibody Conjugate
- (6) One vial of Chromogen (TMB) Solution, which is tetramethylbenzidine (TMB) in citrate/phosphate buffer with hydrogen peroxide
- (7) One vial of concentrated Wash Solution which contains phosphate buffered saline with non-ionic detergent
- (8) One bottle of Stopping Solution which contains 1N sulphuric acid

Assay Procedure

- (1) All samples (sera only) and reagents are brought to room temperature which usually takes 35 minutes and mixed well by vortexing. The Wash Solution is diluted by adding 1500 ml of distilled de-ionized water. It is important to note that crystals may form in the wash solution at cold temperature and will re-dissolve upon warming to room temperature.
- (2) The microplate wells are marked for each samples, standards and controls. Samples, standards, and controls are assayed in duplicate.
- (3) Using a semi-automatic pipette, 50 µl of the Incubation Buffer is added to each well.
- (4) 50 µl of each test samples, NSE standards or NSE control is added to the appropriate well. In order to ensure standard curve consistency, the following order of addition is carried out: (i) test samples, (ii) NSE standards, and (c) NSE controls.
- (5) Cover the microwells with an adhesive plate cover and incubate for 15 minutes on an orbital microplate shaker at room temperature.
- (6) Wash each microwell three times with 340 µl of the Wash Solution using a microplate washer. Blot dry by inverting plate on absorbent paper after each wash.

- (7) 100 μ l of the Peroxidase-antibody Conjugate solution is added to each well.
- (8) Incubate the wells for 15 minutes at room temperature on an orbital microplate shaker.
- (9) Wash each of the microwells three times with 340 μ l of the Wash Solution. Blot dry by inverting the plate on absorbent paper.
- (10) 100 μ l of the TMB solution is added to each of the wells and incubate in the dark at room temperature for 15 minutes.
- (11) 100 μ l of the Stopping Solution is added to each well and the absorbance of the solution in the microwells is read using a microplate reader at 450 nm.

Calculation of Results

- (1) The mean absorbance for each standard, control or test subject serum is calculated.
- (2) The mean absorbance reading for each of the standards is plotted against the NSE concentration (ng/ml).
- (3) The best fitting standard curve is then drawn through the mean of the duplicate points.
- (4) The NSE concentrations of the test subject sera and controls are determined by interpolation from the standard curve.

Nexus Dx™ S-100 Test Kit (Synx Pharma Inc.)

Reagents Supplied

- (1) 96 microtitration wells coated with monoclonal anti-S100 protein antibody.
- (2) Five vials of S-100 protein standards containing 0, 0.02, 0.1, 0.4, and 1.6 ng/ml of S-100 protein.
- (3) One vial of Incubation Buffer.
- (4) Three vials of S-100 protein control serum of low, medium and high levels of S-100 protein.
- (5) One vial of Detection Antibodies (anti-S-100 polyclonal antibodies).
- (6) One vial of goat anti-rabbit immunoglobulins labelled with horseradish peroxidase (Peroxidase Conjugate).
- (7) One vial of concentrated Wash Solution which contains phosphate buffered saline with non-ionic detergent.
- (8) One vial of Chromogen Solution which contains tetramethylbenzidine (TMB) in citrate/phosphate buffer with hydrogen peroxide
- (9) One bottle of Stopping Solution containing 1N sulphuric acid.

Assay Procedures

- (1) All specimens and reagents are brought to room temperature, which usually takes 35 minutes, and mixed well by vortexing. The concentrated Wash Solution is diluted by adding 1500 ml of distilled de-ionised water.
- (2) The microwells are marked for each sample, standards or controls. Samples, standards and controls are assayed in duplicate.
- (3) 75 µl of the Incubation Buffer is added to each well using a semi-automatic dispenser.
- (4) Using a precision micropipette, 25 µl of each test serum sample, S-100 standard and control is added to the appropriate microwell. In order to ensure standard curve consistency, the following order of addition is carried out: (i) test samples, (ii) S-100 standards, and (iii) S-100 controls.
- (5) The microwells are covered using an adhesive plate cover and incubated at room temperature for 15 minutes on an orbital microplate shaker.
- (6) Each microwell is washed three times with 340 µl of the diluted Wash Solution. Blot dry by inverting the plate on absorbent paper after each wash.

- (7) 100 µl of the Detection Antibody Solution is added. The wells are covered by an adhesive plate cover and incubated for 15 minutes at room temperature on an orbital microplate shaker.
- (8) Each microwell is washed three times with 340 µl of the diluted Wash Solution and blotted dry by inverting the plate on absorbent paper after each wash.
- (9) 100 µl of the Peroxidase Conjugate is added to each microwell and incubated for 10 minutes at room temperature on an orbital microplate shaker after being covered by an adhesive plate cover.
- (10) Each microwell is washed three times with 340 µl of the diluted Wash Solution and blotted dry after each wash on absorbent paper.
- (11) 100 µl of the Chromogen Solution is added to each well before being incubated in the dark for 5 minutes at room temperature on an orbital microplate shaker.
- (12) 100 µl of the Stopping Solution is added to each well and the absorbance of the solution in the microwells is read at 450 nm using a microplate reader.

Calculation of Results

- (1) The mean absorbance for each standard, control or test subject serum is calculated.
- (2) The mean absorbance reading for each of the standards is plotted against the S-100 concentration in ng/ml.
- (3) The best fitting standard curve is drawn through the mean of the duplicate points.
- (4) The S-100 concentrations of the test subject sera and controls is determined by interpolation from the standard curve.

Human sL-Selectin Immunoassay (R&D Systems)

Reagent Supplied

- (1) 96 well microplate coated with a mouse monoclonal antibody to human sL-Selectin.
- (2) Six vials of recombinant human sL-Selectin standards with blue dye and preservative. The concentrations of sL-Selectin are shown on the vial labels and vary between kits.
- (3) Three bottles of Sample Diluent which is a buffered protein base with blue dye and preservative.
- (4) One vial of sL-Selectin Conjugate which contains sheep polyclonal antibody to recombinant human sL-Selectin conjugated to horseradish peroxidase in buffer, with red dye and preservative.
- (5) One vial of sL-Selectin Control containing lyophilized human serum with sL-Selectin. The concentration of the control should fall within the range specified on the vial label.
- (6) One bottle of concentrated Wash Buffer which is a 25-fold concentrated buffered surfactant with preservative.
- (7) One bottle of Substrate (stabilized tetramethylbenzidine solution).
- (8) One bottle of Stop Solution (acid solution)
- (9) Plate sealers are included.

Sample Preparation

Both serum and plasma samples can be used with this assay but required to be diluted 100 folds with the Sample Diluent. For this particular children TBI study, the plasma samples are used to determine the sL-Selectin concentrations. Plasma samples are thawed to room temperature which usually takes 35 minutes. 5 µl of the plasma samples are added to 495 µl of the Sample Diluent and mixed by vortexing.

Reagent Preparation

- (1) All reagents are bought to room temperature before use.
- (2) 20 ml of the concentrated Wash Buffer is diluted with 480 ml of distilled de-ionised water. Crystals may be found in the cold Wash Buffer and will re-dissolve upon warming to room temperature and gentle mixing.
- (3) The sL-Selectin Control is reconstituted with 500 µl of distilled de-ionised water immediately before use and allowed to sit at room temperature for at least 10 minutes. The Control is further diluted 100 folds with Sample Diluent prior to assay (10 µl of Control is diluted with 990 µl of Sample Diluent).

Assay Procedure

- (1) The microwells are marked for each standards, control or test samples. All samples, standards, and control are assayed in duplicate.
- (2) 100 µl of Standards, diluted sL-Selectin, and diluted sample is added to the appropriate microwells. Samples addition is uninterrupted and completed within 15 minutes.
- (3) The plate is covered with the provided plate sealer and incubated at room temperature for 1 hour on an orbital microplate shaker.
- (4) 100 µl of the sL-Selectin Conjugate is added to each well with sufficient force to ensure mixing.
- (5) The plate is covered with a new plate sealer and incubated at room temperature for 30 minutes on an orbital microplate shaker.
- (6) Each well is decanted and washed six times with 400 µl of the Wash buffer dispensed by a multi-channel pipette. Complete removal of liquid after each wash is ensured and after the last wash, the plate is blotted dry on clean absorbent paper.
- (7) 100 µl of the Substrate is added to each well. The plate is covered before being incubated in room temperature for 30 minutes on an orbital microplate shaker.
- (8) 100 µl of the Stop Solution is added to each well and the optical density of each well is determined immediately using a microplate reader set at 450 nm, with wavelength correction set at 620 nm.

Calculation of Result

- (1) The mean absorbance values for each set of duplicate Standards are calculated.
- (2) A standard curve is created by reducing the data using Gensis which is a computer software capable of generating a four parameter logistic (4-PL) curve-fit.
- (3) The concentration of each unknown diluted sample is determined by calculating the concentration of sL-Selectin corresponding to the mean absorbance from the standard curve. The concentration determined from the standard curve is then multiplied by the dilution factor to give the actual concentration.

Endothelin (1-21) Test Kit (Biomedica)

Reagent Supplied

- (1) 96 microtitration wells (12 x 8 well microtiter strip in one stripholder) coated with a polyclonal rabbit anti-Endothelin antibody.
- (2) One vial of 10x concentrated Washing Buffer (100 ml).
- (3) One bottle of ready to use Assay Buffer (100 ml).
- (4) One vial of Detection Antibody (Green cap) containing monoclonal mouse anti-Endothelin antibody lyophilized with green dye.
- (5) Six vials of Standards (White caps) synthetic human Endothelin-1 (1-21) in lyophilized human plasma ranging from 0 to 10 fmol/ml. The concentrations after reconstitution are stated on the label.
- (6) Two vials of Controls (Yellow caps) containing synthetic human Endothelin-1 (1-21) in lyophilized human plasma. Concentration after reconstitution are stated on the label.
- (7) One bottle of ready to use Conjugate containing anti-mouse Ig G antibody conjugated to horseradish peroxidase.
- (8) One vial of Substrate which contains ready to use TMB solution.
- (9) One vial of Stop Solution which is ready to use.
- (10) One bottle of PAA (Precipitating Agent Additive) which requires 80 ml of acetone p.a. before use.
- (11) One bottle of PB-Buffer (Protocol B-Buffer).
- (12) One vial of extra high plasma standard (Amber vial).
- (13) Two self-adhesive plastic films

Reagent and Sample Preparation

Since the plasma samples used in this study are EDTA-plasma samples, the protocol for direct measurement of Endothelin in human EDTA-plasma samples is used.

- (1) Test samples are thawed at room temperature for 35 minutes and mixed well by vortexing prior to assay.
- (2) 1.5 ml of the Assay Buffer is added to each of the Standards (0 – 5) and left in room temperature for 30 minutes.
- (3) 1.5 ml of the Assay Buffer is added to each of the Controls and allowed to stand in room temperature for 30 minutes before use.

- (4) 5.5 ml of the Assay Buffer is added to the Detection Antibody which is left to stand in room temperature for 30 minutes.
- (5) Positions for blanks, standards, controls and samples are marked on the protocol sheet supplied.
- (6) The concentrated Washing Buffer is diluted by adding 900 ml of distilled de-ionised water. The solution is mixed well without the formation of foam. Crystals in the concentrated buffer is dissolved upon re-warming in room temperature.
- (7) 200 µl of standards, controls, and samples are introduced into the appropriate wells.
- (8) 50 µl of the Detection Antibody is added to all wells except the blanks.
- (9) The plate is covered and incubated overnight (16-24 hours) at room temperature on an orbital microplate shaker. It is vital that the wells are sealed with the film to prevent evaporation.
- (10) The contents of the wells are discarded and each well is washed five times with at least 300 µl diluted Washing Buffer. The plate is blotted dry on absorbent paper after each wash.
- (11) 200 µl of the Conjugate is added to all wells. The plate is sealed with a new plastic film and incubated for three hours at 37°C without shaking. If an incubator/shaker is available, the incubation time can be reduced to one hour.
- (12) The contents of the wells are discarded and each well is washed five times with at least 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (13) 200 µl of the Substrate is added to each wells and the plate is incubated at room temperature in the dark for 30 minutes on an orbital microplate shaker.
- (14) 50 µl of the Stop Solution is added to the wells. Absorption is determined immediately using a microplate reader at 450 nm against a correction wavelength of 690 nm as reference.

Calculation of Results

- (1) The mean absorbance values for each set of the duplicate Standards are calculated.
- (2) A standard curve, which is expected to be non-linear, is created by using Genesis to reduce the data and generate a four parameter logistic (4-PL) curve-fit.

- (3) The concentration of each unknown diluted sample is determined by calculating the concentration of Endothelin corresponding to the mean absorbance from the standard curve.

SICAM-1 ELISA Kit (Diacalone Research)

Reagents Supplied

- (1) 96 wells microtiter plate coated with a monoclonal antibody specific for ICAM-1.
- (2) Two plastic covers
- (3) Two vials of Standards (8ng/ml) which required to be reconstituted.
- (4) Two vials of Controls which require reconstitution.
- (5) One vial of 10x concentrated Standard Diluent Buffer which requires dilution with distilled water.
- (6) One vial of Biotinylated anti-sICAM-1 which needs to be diluted with biotinylated antibody diluent.
- (7) One vial of ready to use Biotinylated Antibody Diluent.
- (8) Two vials of Streptaviin-HRP which requires to be re-constitute with HRP-Diluent. Further dilutions are required prior to assay.
- (9) One vial of ready to use HRP-Diluent
- (10) One vial of 200x concentrated Washing Buffer. Dilution in distilled water is required prior to use.
- (11) One vial of Chromogen TMB which is ready to use.
- (12) One vial of ready to use H₂SO₄: Stop Reagent.

Preparation of Reagents & Samples

Both serum and plasma samples are suitable for use with this ELISA kit. For the purpose of this study, frozen plasma samples are used.

- (1) 10 ml of the 10x concentrated Standard Buffer Diluent is diluted with 990 ml of distilled de-ionised water and allowed to stand in room temperature for 30 minutes prior to use.
- (2) Tests samples are thawed in room temperature for 35 minutes. 5 µl of each sample is diluted with 495 µl of the diluted Standard Diluent i.e 100x dilution.
- (3) Reconstitution of the Standard is done according to the instruction on the vial to give a stock solution of 8 ng/ml of sICAM-1. The solution is allowed to stand for 5 minutes with gentle swirling prior to further dilutions.
- (4) Control is reconstituted as per instruction on the vial and left to stand for 5 minutes with gentle swirling before distribution into the control wells.

- (5) 10 ml of the Washing buffer is added to 1990 ml of distilled de-ionised water at room temperature.

Assay Procedure

- (1) The position of standards, controls and samples are marked. Each sample, standard, blank, and control samples are assayed in duplicate.
- (2) 100 μ l of the diluted Standard Diluent is added to standard wells labelled B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2. 200 μ l of the reconstituted Standard is added to the wells labelled A1 and A2. 100 μ l of the Standard in wells A1 and A2 is transferred into wells B1 and B2. The contents are mixed by repeated aspiration and ejections. The procedure is repeated from the wells B1, B2 to wells C1, C2, and from wells C1, C2 to D1, D2 and so on creating two parallel rows of sICAM-1 standard dilutions ranging from 8 ng/ml to 0.25 ng/ml. 100 μ l of the content of the last microwells F1 and F2 is discarded. Care is taken to ensure the inner surface of the microwells are not scratched.
- (3) 100 μ l of the Standard Diluent is added to each of the blank wells (G1 and G2).
- (4) 100 μ l of the reconstituted Control is added into the Control wells (H1 and H2) and 100 μ l of each test sample is added to the appropriate well.
- (5) 240 μ l of the Biotinylated anti-sICAM-1 is diluted with 6360 μ l of the Biotinylated Antibody Diluent immediately before use. 50 μ l of the diluted Biotinylated Antibody is added to all wells.
- (6) The plate is covered with the plastic film and incubated at room temperature for 1 hour on an orbital microplate shaker.
- (7) The fluid in each well is discarded. Each well is then washed three times with 300 μ l of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (8) 500 μ l of the HRP Diluent is added to the Streptavidin-HRP to make up a stock solution. 150 μ l of this stock solution is further diluted with 10 ml of HRP Diluent immediate prior to assay. 100 μ l of the diluted Streptavidin-HRP is added to each wells including the blank wells before being covered and incubated at room temperature for 30 minutes on an orbital microplate shaker.
- (9) The fluid in each well is discarded. Each well is then washed three times with 300 μ l of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.

- (10) 100 µl of the TMB substrate solution is added to each well including the blank wells. Incubation in the dark for 15 minutes is done at room temperature on an orbital microplate shaker.
- (11) 100 µl of the H₂SO₄: Stop Reagent is added quickly to each well. Absorbance of each well is read immediately using a microplate reader at 450 nm against a reference wavelength of 620 nm.

Calculation of Result

- (1) The mean absorbance for each set of the standard duplicate is calculated.
- (2) A linear standard curve is generated by plotting the average absorbance against the corresponding sICAM-1 standard concentration.
- (3) The sICAM-1 concentration in each sample is determined by extrapolating OD values to sICAM-1 concentration using the standard curve. The actual concentration of sICAM-1 in each sample is obtained by multiplying the dilution factor (100x).

IL-10 ELISA Kit (Diacclone Research)

Reagents Supplied

- (1) 96 wells microtiter plate coated with a monoclonal antibody specific for IL-10.
- (2) Two plastic covers
- (3) Two vials of Standards (400pg/ml) which require reconstitution.
- (4) Two vials of Controls which require reconstitution.
- (5) One vial of 10x concentrated Standard Diluent Buffer which requires dilution with distilled water.
- (6) One vial of ready to use Standard Diluent: Human Serum.
- (7) One vial of Biotinylated anti-IL-10 which needs to be diluted with biotinylated antibody diluent.
- (8) One vial of ready to use Biotinylated Antibody Diluent.
- (9) Two vials of Streptavidin-HRP which requires to be re-constituted with HRP-Diluent. Further dilutions are required prior to assay.
- (10) One vial of ready to use HRP-Diluent
- (11) One vial of 200x concentrated Washing Buffer. Dilution in distilled water is required prior to use.
- (12) One vial of Chromogen TMB which is ready to use.
- (13) One vial of ready to use H₂SO₄: Stop Reagent.

Preparation of Reagents and Samples

Both plasma and serum samples are suitable for use with this assay kit. For the purpose of this study, EDTA-tracalon plasma samples are used.

- (1) Test specimens are thawed at room temperature for 35 minutes.
- (2) Standard is reconstituted with Standard Diluent: Human Serum as per instruction on the vial to provide a stock solution of 400 pg/ml. This is allowed to stand in room temperature for 5 minutes prior to assay.
- (3) Control is reconstituted with Standard Diluent: Human Serum in accordance with the instruction on the vial. This is left in room temperature for 5 minutes before use.

- (4) 10 ml of the concentrated Washing Buffer is diluted with 1990 ml of distilled de-ionised water and left in room temperature at least 30 minutes before assay.

Assay Procedure

- (1) The position of standards, controls and samples are marked. Each sample, standard, blank, and control samples are assayed in duplicate.
- (2) 100 µl of the diluted Standard Diluent: Human Serum is added to standard wells labelled B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2.
- (3) 200 µl of the reconstituted Standard is added to the wells labelled A1 and A2.
- (4) 100 µl of the Standard in wells A1 and A2 is transferred into wells B1 and B2. The contents are mixed by repeated aspiration and ejections.
- (5) The procedure is repeated from the wells B1, B2 to wells C1, C2, and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-10 standard dilutions ranging from 400 pg/ml to 12.5 pg/ml. 100 µl of the content of the last microwells F1 and F2 is discarded. Care is taken to ensure the inner surface of the microwells are not scratched.
- (6) 100 µl of the Standard Diluent: Human Serum is added to each of the blank wells (G1 and G2).
- (7) 100 µl of the reconstituted Control is added into the Control wells (H1 and H2) and 100 µl of each test sample is added to the appropriate well.
- (8) 240 µl of the Biotinylated anti-IL-10 is diluted with 6360 µl of the Biotinylated Antibody Diluent immediately before use. 50 µl of the diluted Biotinylated Antibody is added to all wells.
- (9) The plate is covered with the plastic film and incubated at room temperature for 2 hours on an orbital microplate shaker.
- (10) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (11) 500 µl of the HRP Diluent is added to the Streptavidin-HRP to make up a stock solution. 150 µl of this stock solution is further diluted with 10 ml of HRP Diluent immediate prior to assay. 100 µl of the diluted Streptavidin-HRP is added to each well including the blank wells before being covered and incubated at room temperature for 30 minutes on an orbital microplate shaker.

- (12) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (13) 100 µl of the TMB substrate solution is added to each well including the blank wells. Incubation in the dark for 12 minutes is done at room temperature on an orbital microplate shaker.
- (14) 100 µl of the H₂SO₄: Stop Reagent is added quickly to each well. Absorbance of each well is read immediately using a microplate reader at 450 nm against a reference wavelength of 620 nm.

Calculation of Result

- (1) The mean absorbance for each set of the standard duplicate is calculated.
- (2) The average absorbance is plotted against the corresponding IL-10 standard concentration and the best fitting curve is drawn through the mean of the duplicate points using 4-PL protocol.
- (3) The IL-10 concentration in each sample is determined by extrapolating OD values to IL-10 concentration using the standard curve.

IL-6 ELISA Kit (Diacalone Research)

Reagents Supplied

- (1) 96 wells microtiter plate coated with a monoclonal antibody specific for IL-6.
- (2) Two plastic covers
- (3) Two vials of Standards (200 pg/ml) which require reconstitution.
- (4) Two vials of Controls which require reconstitution.
- (5) One vial of 10x concentrated Standard Diluent Buffer which requires dilution with distilled water.
- (6) One vial of ready to use Standard Diluent: Human Serum.
- (7) One vial of Biotinylated anti-IL-6 which needs to be diluted with biotinylated antibody diluent.
- (8) One vial of ready to use Biotinylated Antibody Diluent.
- (9) Two vials of Streptavidin-HRP which requires to be re-constituted with HRP-Diluent. Further dilutions are required prior to assay.
- (10) One vial of ready to use HRP-Diluent
- (11) One vial of 200x concentrated Washing Buffer. Dilution in distilled water is required prior to use.
- (12) One vial of Chromogen TMB which is ready to use.
- (13) One vial of ready to use H₂SO₄: Stop Reagent.

Preparation of Reagents and Samples

Both plasma and serum samples are suitable for use with this assay kit. For the purpose of this study, EDTA-tracalon plasma samples are used.

- (1) Test specimens are thawed at room temperature for 35 minutes.
- (2) Standard is reconstituted with Standard Diluent: Human Serum as per instruction on the vial to provide a stock solution of 200 pg/ml. This is allowed to stand in room temperature for 5 minutes prior to assay.
- (3) Control is reconstituted with Standard Diluent: Human Serum in accordance with the instruction on the vial. This is left in room temperature for 5 minutes before use.

- (4) 10 ml of the concentrated Washing Buffer is diluted with 1990 ml of distilled de-ionised water and left in room temperature at least 30 minutes before assay.

Assay Procedure

- (1) The position of standards, controls and samples are marked. Each sample, standard, blank, and control samples are assayed in duplicate.
- (2) 100 µl of the diluted Standard Diluent:Human Serum is added to standard wells labelled B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2.
- (3) 200 µl of the reconstituted Standard is added to the wells labelled A1 and A2.
- (4) 100 µl of the Standard in wells A1 and A2 is transferred into wells B1 and B2. The contents are mixed by repeated aspiration and ejections.
- (5) The procedure is repeated from the wells B1, B2 to wells C1, C2, and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-10 standard dilutions ranging from 200 pg/ml to 6.25 pg/ml. 100 µl of the content of the last microwells F1 and F2 is discarded. Care is taken to ensure the inner surface of the microwells are not scratched.
- (6) 100 µl of the Standard Diluent: Human Serum is added to each of the blank wells (G1 and G2).
- (7) 240 µl of the Biotinylated anti-IL-6 is diluted with 6360 µl of the Biotinylated Antibody Diluent immediately before use. 50 µl of the diluted Biotinylated Antibody is added to all wells.
- (8) The plate is covered with the plastic film and incubated at room temperature for 1 hour on an orbital microplate shaker.
- (9) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (10) 500 µl of the HRP Diluent is added to the Streptavidin-HRP to make up a stock solution. 150 µl of this stock solution is further diluted with 10 ml of HRP Diluent immediate prior to assay. 100 µl of the diluted Streptavidin-HRP is added to each well including the blank wells before being covered and incubated at room temperature for 30 minutes on an orbital microplate shaker.
- (11) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.

- (12) 100 µl of the TMB substrate solution is added to each well including the blank wells. Incubation in the dark for 12 minutes is done at room temperature on an orbital microplate shaker.
- (13) 100 µl of the H₂SO₄: Stop Reagent is added quickly to each well. Absorbance of each well is read immediately using a microplate reader at 450 nm against a reference wavelength of 620 nm.

Calculation of Result

- (1) The mean absorbance for each set of the standard duplicate is calculated.
- (2) The average absorbance is plotted against the corresponding IL-6 standard concentration and the best fitting curve is drawn through the mean of the duplicate points using 4-PL protocol.
- (3) The IL-6 concentration in each sample is determined by extrapolating OD values to IL-6 concentration using the standard curve.

In samples with concentration below detection level (<6.25 pg/ml), the High Sensitivity Human IL-6 ELISA Kit (Diacclone Research) is used to repeat the assay. This provides a detection range from 1.56 pg/ml to 50 pg/ml. The assay protocol for the High Sensitivity Human IL-6 ELISA Kit is identical to that of IL-6 ELISA Kit with the only difference being the concentration of the Standard provided in the High Sensitivity Kit is 50 pg/ml.

IL-8 ELISA Kit (Diacclone Research)

Reagents Supplied

- (1) 96 wells microtiter plate coated with a monoclonal antibody specific for IL-8.
- (2) Two plastic covers
- (3) Two vials of Standards (2000 pg/ml) which require reconstitution.
- (4) Two vials of Controls which require reconstitution.
- (5) One vial of 10x concentrated Standard Diluent Buffer which requires dilution with distilled water.
- (6) One vial of ready to use Standard Diluent: Human Serum.
- (7) One vial of Biotinylated anti-IL-8 which needs to be diluted with biotinylated antibody diluent.
- (8) One vial of ready to use Biotinylated Antibody Diluent.
- (9) Two vials of Streptavidin-HRP which requires to be re-constituted with HRP-Diluent. Further dilutions are required prior to assay.
- (10) One vial of ready to use HRP-Diluent
- (11) One vial of 200x concentrated Washing Buffer. Dilution in distilled water is required prior to use.
- (12) One vial of Chromogen TMB which is ready to use.
- (13) One vial of ready to use H₂SO₄: Stop Reagent.

Preparation of Reagents and Samples

Both plasma and serum samples are suitable for use with this assay kit. For the purpose of this study, EDTA-tracalon plasma samples are used.

- (1) Test specimens are thawed at room temperature for 35 minutes.
- (2) Standard is reconstituted with Standard Diluent: Human Serum as per instruction on the vial to provide a stock solution of IL-8 with concentration of 2000 pg/ml. This is allowed to stand in room temperature for 5 minutes prior to assay.
- (3) Control is reconstituted with Standard Diluent: Human Serum in accordance with the instruction on the vial. This is left in room temperature for 5 minutes before use.

- (4) 10 ml of the concentrated Washing Buffer is diluted with 1990 ml of distilled de-ionised water and left in room temperature at least 30 minutes before assay.

Assay Procedure

- (1) The position of standards, controls and samples are marked. Each sample, standard, blank, and control samples are assayed in duplicate.
- (2) 100 µl of the diluted Standard Diluent: Human Serum is added to standard wells labelled B1, B2, C1, C2, D1, D2, E1, E2, F1, and F2.
- (3) 200 µl of the reconstituted Standard is added to the wells labelled A1 and A2.
- (4) 100 µl of the Standard in wells A1 and A2 is transferred into wells B1 and B2. The contents are mixed by repeated aspiration and ejections.
- (5) The procedure is repeated from the wells B1, B2 to wells C1, C2, and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-8 standard dilutions ranging from 2000 pg/ml to 62.5 pg/ml. 100 µl of the content of the last microwells F1 and F2 is discarded. Care is taken to ensure the inner surface of the microwells are not scratched.
- (6) 100 µl of the Standard Diluent: Human Serum is added to each of the blank wells (G1 and G2).
- (7) 240 µl of the Biotinylated anti-IL-8 is diluted with 6360 µl of the Biotinylated Antibody Diluent immediately before use. 50 µl of the diluted Biotinylated Antibody is added to all wells.
- (8) The plate is covered with the plastic film and incubated at room temperature for 1 hour on an orbital microplate shaker.
- (9) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.
- (10) 500 µl of the HRP Diluent is added to the Streptavidin-HRP to make up a stock solution. 150 µl of this stock solution is further diluted with 10 ml of HRP Diluent immediate prior to assay. 100 µl of the diluted Streptavidin-HRP is added to each well including the blank wells before being covered and incubated at room temperature for 30 minutes on an orbital microplate shaker.
- (11) The fluid in each well is discarded. Each well is then washed three times with 300 µl of the diluted Washing Buffer. The plate is blotted dry after each wash on absorbent paper.

- (12) 100 µl of the TMB substrate solution is added to each well including the blank wells. Incubation in the dark for 12 minutes is done at room temperature on an orbital microplate shaker.
- (13) 100 µl of the H₂SO₄: Stop Reagent is added quickly to each well. Absorbance of each well is read immediately using a microplate reader at 450 nm against a reference wavelength of 620 nm.

Calculation of Result

- (1) The mean absorbance for each set of the standard duplicate is calculated.
- (2) The average absorbance is plotted against the corresponding IL-8 standard concentration and the best fitting curve is drawn through the mean of the duplicate points using 4-PL protocol.

The IL-8 concentration in each sample is determined by extrapolating OD values to IL-8 concentration using the standard curve.

APPENDIX III

PRESENTATIONS

APPENDIX III-a
SUMMARY LIST OF PRESENTATIONS

Oral Presentations

- (1) Scottish National Paediatric Research Day, May 2002, Edinburgh. 'Is cerebral atrophy the cause of microcephaly after Non-accidental Head Injury?'
- (2) The South East Scotland Intensive Care Group Annual Meeting, July 2002, Edinburgh. 'High frequency oscillatory ventilation (HFOV): Is it an alternative ventilatory method in children with head injury and respiratory failure?'
- (3) The European Conference on Shaken Baby Syndrome, May 2003, Edinburgh. 'Is neuronal loss progressive after non-accidental head injury?'
- (4) Paediatric Intensive Care Society Meeting, September 2003, Bristol. 'Nursing care procedures during the acute management of children with traumatic brain injury: Are we worsening the degree and duration of secondary physiological insults?'
- (5) The 14th European Society of Paediatric and Neonatal Intensive Care Medical and Nursing Annual Congress, November 2003, Athens. 'Critical cerebral perfusion pressure thresholds in paediatric traumatic brain injury.'
- (6) The 14th European Society of Paediatric and Neonatal Intensive Care Medical and Nursing Annual Congress, November 2003, Athens. 'Effects of nursing care procedures on the degree and duration of age-specific secondary physiological insults in the acute management of children with traumatic brain injury.'
- (7) The 8th Spring Meeting of the Royal College of Paediatrics and Child Health, March 2004, York. 'Age related critical cerebral perfusion pressure thresholds in paediatric traumatic brain injury.'
- (8) The 9th Congress of the EMN, May 2004, Romania. 'Correlations of the neurochemical markers, age-specific secondary physiological derangements and outcome in children with traumatic brain injury.'
- (9) The 12th International Symposium on Intracranial Pressure and Brain Monitoring, August 2004, Hong Kong. 'Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure-time index.'
- (10) The combined 15th European Society of Paediatric and Neonatal Intensive Care Medical and Nursing Annual Congress and the Annual Paediatric Intensive Care Society Meeting, September 2004, London. 'Influence of Apolipoprotein E genetic polymorphisms on age-specific secondary physiological derangement and outcome following childhood traumatic brain injury.'

- (11) The British Paediatric Neurology Association Annual Meeting January 2005, London. 'Apolipoprotein E genetic polymorphisms and childhood traumatic brain injury: influence on age-specific secondary physiological derangements and outcome.' The British Paediatric Neurology Association Annual Meeting January 2005, London. 'The value of mean cerebral perfusion pressure (CPP), mean intracranial pressure (ICP), and mean 'mean arterial pressure' (MAP) from successive 6-hour epochs, as a predictor of outcome after children's head injury.'
- (12) The 9th Spring Meeting of the Royal College of Paediatrics and Child Health, April 2005, York. 'Variation in cerebral perfusion pressure insults among apolipoprotein E genetic polymorphisms and associations with outcome following childhood traumatic brain injury.' (Oral poster presentation)
- (13) The 9th Spring Meeting of the Royal College of Paediatrics and Child Health, April 2005, York. 'Regional retrieval teams for head injured children: How will this affect A&E?'
- (14) The 17th Annual Congress of the European Academy of Childhood Disability, November 2005, Monaco. 'Genetic and biochemical influence on outcome of childhood brain trauma.' (Invited Speaker)
- (15) The Annual Scientific Meeting of the Faculty of Accident and Emergency Medicine, November 2005, Edinburgh. 'Variation in cerebral perfusion pressure insults among apolipoprotein E genetic polymorphisms and associations with outcome following childhood traumatic brain injury.'
- (16) 'The 3rd Paediatric Neurosciences Benchmarking Group Meeting, March 2006, Bristol. 'Influence of age-related cerebral perfusion pressure insults and Apolipoprotein E genetic polymorphisms on the outcome of childhood brain trauma.' (Invited Speaker)
- (17) The 13th Intracranial Pressure and Brain Monitoring Symposium, July 2007. San Francisco. 'Are head injury guidelines changing the outcome of head injured children? A regional investigation.'

Poster Presentations

- (1) BPNA 2002 – British Paediatric Neurology Association Annual Meeting, Newcastle upon Tyne, UK. ‘Variability and inter-relationships of pressure signals in traumatic brain injury (TBI).’
- (2) ESMRN 2002 – The 7th Biennial Meeting of the European Society of Magnetic Resonance in Neuropaediatrics, London, UK. ‘Rapid development of cerebral atrophy following non-accidental head injury (NAHI).’
- (3) The 4th World Congress in Pediatric Intensive Care Medicine (2003), Boston, U.S.A. ‘High frequency oscillatory ventilation (HFOV) in the management of refractory intracranial hypertension in children with severe traumatic brain injury (TBI) and concomitant lung pathology.’
- (4) The 4th World Congress in Pediatric Intensive Care Medicine (2003), Boston, U.S.A. ‘Neurochemical markers, age-specific secondary physiological derangements and outcome in children with traumatic brain injury.’
- (5) The 4th World Congress in Pediatric Intensive Care Medicine (2003), Boston, U.S.A. ‘Contribution of nursing care activities and therapy procedures to secondary physiological derangements in critically ill children with traumatic brain injury (TBI).’
- (6) The 14th European Society of Paediatric and Neonatal Intensive Care Medical and Nursing Annual Congress (2003), Athens, Greece. ‘Does high frequency oscillatory ventilation have a role in reducing secondary physiological insults during the acute management of children with concomitant traumatic brain injury and acute lung injury? – A report of two cases.’
- (7) BPNA 2004 - British Paediatric Neurology Association Annual Meeting, Sheffield, UK. ‘Critical cerebral perfusion pressure thresholds in paediatric traumatic brain injury.’

APPENDIX III-b

SAMPLE PRESENTATIONS

SAMPLE PRESENTATION 1

AGE-RELATED CRITICAL CEREBRAL PERFUSION PRESSURE THRESHOLDS IN PAEDIATRIC TRAUMATIC BRAIN INJURY

**First Prize in Oral Presentation,
The 8th Royal College of Paediatrics & Child Health Annual Meeting
(March 2004)**

SAMPLE PRESENTATION 2

VARIATION IN CEREBRAL PERFUSION PRESSURE INSULT AMONG APOLIPOPROTEIN E GENETIC POLYMORPHISMS AND ASSOCIATIONS WITH OUTCOME FOLLOWING CHILDHOOD TRAUMATIC BRAIN INJURY

**Roderick Little Prize for Oral Presentation
Annual Faculty of Emergency Medicine Congress
(November 2005)**



Age-related Critical CPP Thresholds in Paediatric Traumatic Brain Injury

T.Y.M. Lo^{1&2}, P.A. Jones¹, I.R. Chambers³,
A. Clark³, J. Croft³, G. Wilson³, B. Fulton³,
R. Forsyth³, P.J.D. Andrews¹,
A.D. Mendelow³, R.A. Minns^{1&2}

¹University of Edinburgh
²Royal Hospital for Sick Children, Edinburgh
³Newcastle General Hospital

In children, duration of age-specific abnormal CPP predict outcome

- Dead vs alive ($p = 0.003$)
- 'Poor' vs 'independent' ($p = 0.004$)

(Jones et al. British Journal of Neurosurgery 2003; 17(1):29-39.)

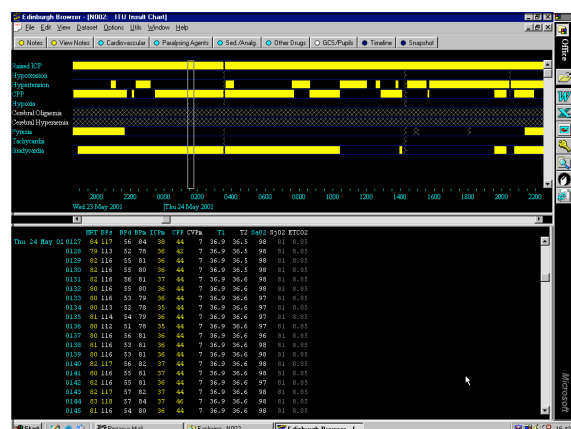
Aims

In children with TBI

1. Quantify the physiological derangement
 - Using a cumulative pressure-time index (CP-T)
2. Correlate physiological derangement (mean values & CP-T) with outcome
3. Determine 'critical age-related CPP thresholds' (Abnormal physiological thresholds)

Patients & Methods

- Prospective observational study
- 79 children
 - 52 boys, 27 girls
 - Severe 58, moderate 19, minor 2
- Two regional centres



Patients & Methods

- Outcome assessment (GOS at 6 months)
 - ‘independent’ vs ‘poor’
- Severity of derangement
 - ROC curve
- Predictors of outcome
 - Univariate & multivariate logistic regression modelling

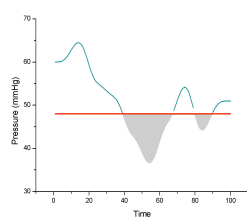
Patients & Methods Critical CPP Thresholds & CP-T

Age	Mean CPP threshold
2-6 years	≤ 48 mmHg
7-10 years	≤ 54 mmHg
11-16 years	≤ 58 mmHg

$$CPT = \sum (CPP_{\text{threshold}} - CPP) \times t_{\text{sample interval}}$$

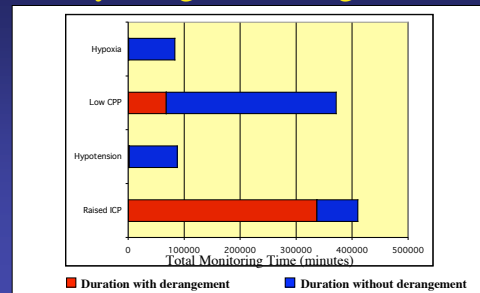
Patients & Methods Cumulative pressure-time index (CP-T)

$$CPT = \sum (CPP_{\text{threshold}} - CPP) \times t_{\text{sample interval}}$$

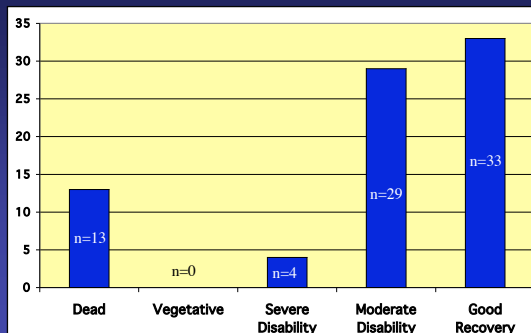


Cumulative area between the threshold and the curve

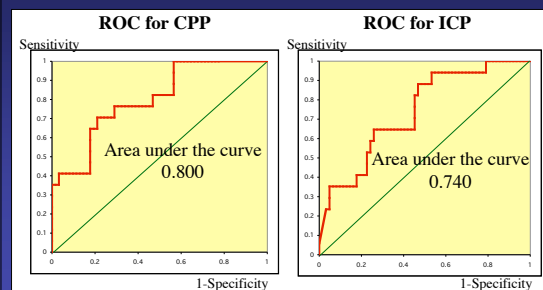
Results - Physiological Derangement



Results - Outcome



Results - ROC Curves



Results - Logistic Regression Modelling

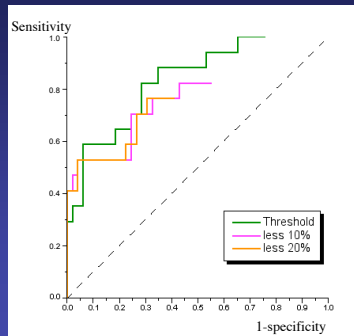
Variables	Univariate Logistic Regression	Final Model
Low CPP	$p=0.0003$	$p=0.0003$
Raised ICP	$p=0.027$	$p=0.131$
ISS	$p=0.036$	$p=0.369$
GCS sumscore	$p=0.005$	$p=0.280$

Results - CP-T & Outcome

Outcome	Mean CP-T Value
Independent	3228 (95% CI 1557, 4898)
Poor	32713 (95% CI 16168, 49258)

Significant difference between the CP-T area product and outcome ($p<0.001$)

ROC Curve for CPP Threshold



Conclusion - 1

In children with traumatic brain injury:

- CPP was the best predictor of outcome
- CP-T was useful in quantifying secondary insults

Conclusion - 2 Critical CPP Thresholds (Abnormal Physiology)

Age	Mean CPP threshold
2 - 6 years	48 mmHg
7 - 10 years	54 mmHg
11 - 16 years	58 mmHg

Variation in cerebral perfusion pressure insults among apolipoprotein E genetic polymorphisms and associations with outcome following childhood traumatic brain injury

Dr. T.Y.M. Lo^{1&2}, Mrs. P.A. Jones^{1&2}, Dr. I.R. Chambers³,
Dr. T.F. Beattie², Ms. J. Croft³, Ms. G. Wilson³,
Dr. R. Forsyth³, Prof. A.D. Mendelow³,
Prof. R.A. Minns^{1&2}



¹Child Life & Health, University of Edinburgh, Edinburgh

²Royal Hospital for Sick Children, Edinburgh

³Newcastle General Hospital, Newcastle upon Tyne



Despite vigilant trauma management & neuro-intensive care, outcome remains diverse after childhood traumatic brain injury

Background APO E Genotypes & Brain Injury

Apolipoprotein E Gene

- Polymorphism in human
 - $\epsilon 2$, $\epsilon 3$, $\epsilon 4$
- Gene product (apo E) & CNS lipid transport system
- In adults, APO E $\epsilon 4$
 - Poor recovery from head injury
(Teasdale et al, *The Lancet* 1997)
(Friedman et al, *Neurology* 1999)
(Teasdale et al, *Brain* 2005)
 - Increased risk for Alzheimer's Disease
(Horsburgh et al, *Neurobiol Aging* 2000)

Aim

To determine:

- (1) The distribution of APO E alleles:
 - Critically ill head injured children
 - Healthy active controls
- (2) The influence of APO E alleles on:
 - Amount of age-specific CPP insult
 - Outcome after childhood TBI

Patients & Methods

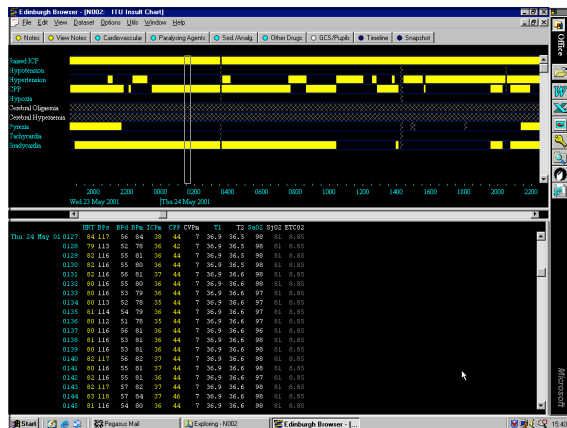


- Prospective case-control study
- 65 critically ill head injured children
- 160 active healthy children (Controls)

Patients & Methods

- Buccal smear for DNA extractions
- APO E genotyping
 - PCR
 - Restriction enzyme (Hha I) digest
 - Metaphor agarose gel electrophoresis





Results: Median CPT for CPP (mmHg.Hr)

	Whole Group	ε2 Present	ε3 Homoz.	ε4 Present	p values
Good Recovery	51.3	35.8	65.5	7.8	p < 0.05
Poor Outcome	107.3	107.3	329.0	49.0	Insuff. no.

Summary - 1

- No difference in distribution of APO E genotypes in active children with / without head injury
- Distribution of APO E genotype (APO E ε2) in active children with / without head injury differs significantly from those previously reported in adults.

Summary - 2

- The possession of **different APO E alleles** are associated with **different amounts of CPP insults** (as measured by CPT).
- Carriers of the **APO E ε4** allele experienced the **least amount of CPP insult**.
- The degree of CPP insult for APO E ε4 possessors with a poor outcome was less than half that expected for the whole group, and at a level where a good recovery should have been expected, **suggesting other mechanisms influenced by the genotype, were responsible for the poor outcome.**

Conclusion

- Better understanding of how APO E genetic polymorphisms affect outcome after brain trauma.
- Development of novel supplemental Rx to current Rx strategy (secondary insult prevention) used in ED & neuro-ICU.

APPENDIX IV

PUBLICATIONS

APPENDIX IV-a

SUMMARY LIST OF PUBLICATIONS

Original Articles

- (1) Jones PA, Minns RA, **Lo TYM**, Andrews PJD, Taylor GS, Ali S. Graphical display of the variability and inter-relationships of pressure signals in children with traumatic brain injury. *Physiological Measurement*. February 2003; 24: 201-211. (doi: 10.1088/0967-3334/24/1/315)
- (2) **Lo TYM**, McPhillips M, Minns RA, Gibson RJ. Cerebral atrophy following shaken impact syndrome and other non-accidental head injury (NAHI). *Pediatric Rehabilitation*. January – March 2003; 6(1): 47-55. (doi: 10.1080/1363849031000109516)
- (3) Jones PA, Chambers IR, **Lo TYM**, Andrews PJD, Chaudhry W, Clark A, Croft J, Forsyth R, Fulton B, Mendelow AD, Wilson G, Minns RA. Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure time index. *Acta Neurochirurgica*. 2005; 95(Supplement): 29 – 32.
- (4) Chambers IR, Jones PA, **Lo TYM**, Forsyth R, Fulton B, Andrews PJD, Mendelow AD, Minns RA. Critical thresholds of intracranial pressure and cerebral perfusion pressure related to age in paediatric head injury. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2006; 77: 234-240. (doi: 1136/jnnp.2005.072215)
- (5) **Lo TYM**, Jones PA, Freeman JA, McFadzean J, Minns RA. The role of High Frequency Oscillatory Ventilation (HFOV) in the management of children with severe traumatic brain injury (TBI) and concomitant lung pathology where conventional ventilation had failed. *Pediatric Critical Care Medicine*. 2008; 9(5): e38-e42.
- (6) **Lo TYM**, Jones PA, Chambers IR, Beattie TF, Forsyth R, Mendelow AD, Minns RA. Modulating effect of Apolipoprotein E polymorphisms on secondary insult and outcome after childhood brain trauma. *Child's Nervous System*. 2009; 25: 47-54. (doi: 10.1007/s00381-008-0723-4)
- (7) Jones PA, Chambers IR, Minns RA, **Lo TYM**, Myles LM, Steers AJW. Are head injury guidelines changing the outcome of head injured children? A regional investigation. *Acta Neurochirurgica (Supplement)*. 2009; (In press).
- (8) **Lo TYM**, Jones PA, Minns RA. Pediatric brain trauma outcome prediction using paired serum levels of inflammatory mediators and brain specific proteins. *Journal of Neurotrauma*. 2009; (In press).

Book Chapter

Minns RA, **Lo TYM**. Head and neck injuries. In *Paediatric Forensic Medicine and Pathology* (2008). Edward Arnold Publishing. Editors: JW Keeling & A Busuttill. .

Abstracts

- (1) Jones PA, Minns RA, **Lo TYM**, Andrews PJD. Variability and inter-relationships of pressure signals in traumatic brain injury (TBI). *Developmental Medicine & Child Neurology*. December 2001; 43(Supplement): 30.

- (2) McPhillips M, **Lo M**, Gibson RJ, Minns RA. Rapid development of cerebral atrophy following shaking injury. *Child's Nervous System*. February 2002; 18(1-2): 105.
- (3) Jones PA, **Lo TYM**, Chambers IR, Wilson G, Mendelow AD, Forsyth R, Fulton B, Andrews PJD, Minns RA. Edinburgh-Newcastle Study: Secondary insults after accidental and non-accidental brain injury in children, and relationship to outcome – Work in progress. *Acta Neurochirurgica*. July 2002; 144(7): A19.
- (4) **Lo TYM**, Jones PA, Freeman J, McFadzean J, Minns RA. High frequency oscillatory ventilation (HFOV) in the management of refractory intracranial hypertension in children with severe traumatic brain injury (TBI) and concomitant lung pathology. *Pediatric Critical Care Medicine*. (Supplement) July 2003; 4(3): A65.
- (5) **Lo TYM**, Jones PA, McFadzean J, Minns RA. Neurochemical markers, age-specific secondary physiological derangements and outcome in children with traumatic brain injury. *Pediatric Critical Care Medicine*. (Supplement) July 2003; 4(3): A173.
- (6) Holmes A, Shaw K, Richardson J, Lawson J, Johnston P, **Lo TYM**, Freeman J. Contribution of nursing care activities and therapy procedures to secondary physiological derangements in critically ill children with traumatic brain injury (TBI). *Pediatric Critical Care Medicine*. (Supplement) July 2003; 4(3): A173.
- (7) **Lo TYM**, Jones PA, Chambers IR, Clark A, Croft J, Wilson G, Fulton B, Forsyth R, Andrews PJD, Mendelow AD, Minns RA. Critical cerebral perfusion pressure thresholds in paediatric traumatic brain injury. *Developmental Medicine & Child Neurology*. January 2004; 46 (Supplement): 11.
- (8) **Lo TYM**, Jones PA, Chambers IR, Clark A, Wilson G, Croft J, Fulton B, Forsyth R, Andrews PJD, Mendelow AD, Minns RA. Age related critical cerebral perfusion pressure thresholds in paediatric traumatic brain injury. *Archives of Disease in Childhood*. April 2004; 89 (Supplement): A26.
- (9) Jones PA, Chambers IR, Andrews PJ, Chaudhry W, Clark A, Croft J, Forsyth R, Fulton B, **Lo TYM**, Mendelow AD, Wilson G, Minns RA. Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure-time index. *Proceedings of the 12th International Symposium on Intracranial Pressure and Brain Monitoring*. August 2004.
- (10) **Lo TYM**, Jones PA, Croft J, Wilson G, Beattie TF, Forsyth R, Mendelow AD, Minns RA. Apolipoprotein E genetic polymorphisms and childhood traumatic brain injury: influence on age-specific secondary physiological derangements and outcome. *Developmental Medicine & Child Neurology*. January 2005; 47 (Supplement): 5.
- (11) Jones PA, Chambers IR, **Lo TYM**, Forsyth R, Fulton B, Mendelow AD, Minns RA. The value of mean cerebral perfusion pressure (CPP), mean intracranial pressure (ICP), and mean 'mean arterial pressure' (MAP) from successive 6-hour epochs, as a predictor of outcome after children's head injury. *Developmental Medicine & Child Neurology*. January 2005; 47 (Supplement): 5.

- (12) **Lo TYM**, Jones PA, Chambers IR, Croft J, Wilson G, Beattie TF, Forsyth R, Mendelow AD, Minns RA. Variation in cerebral perfusion pressure insults among apolipoprotein E genetic polymorphisms and associations with outcome following childhood traumatic brain injury. *Archives of Disease in Childhood*. April 2005; 90 (Supplement): A36.
- (13) Jones PA, Chambers IR, **Lo TYM**, Minns RA. Cerebral perfusion pressure insult characteristics (duration and intensity) which best predict poor outcome after severe childhood traumatic brain injury. *Developmental Medicine and Child Neurology*. January 2006; 48 (Supplement): 3-4.
- (14) **Lo TYM**, Jones PA, Minns RA. Correlations of the neurochemical markers, age-specific secondary physiological derangements and outcome in children with traumatic brain injury. *Acta Neurochirurgica*. May 2006; 148(5):XV.

APPENDIX IV–b

PUBLISHED PAPERS

PAPER 1 (PUBLISHED)

Jones PA, Minns RA, *Lo TYM*, Andrews PJD, Taylor GS, Ali S.

Graphical display of the variability and inter-relationships of pressure signals in children with traumatic brain injury.

Physiological Measurement. February 2003; 24: 201-211.

doi: 10.1088/0967-3334/24/1/315

<http://www.iop.org/EJ/abstract/0967-3334/24/1/315>

Homepage of Physiological Measurement: www.iop.org/journals/pmea

Reproduced with the Publisher's Permissions

Graphical display of variability and inter-relationships of pressure signals in children with traumatic brain injury

P A Jones^{1,2}, R A Minns^{1,2,7}, T Y M Lo^{1,2}, P J D Andrews^{3,4}, G S Taylor⁵ and S Ali^{6,8}

¹ Department of Child Life and Health, University of Edinburgh, Edinburgh EH9 1LF, UK

² Royal Hospital for Sick Children, 9 Sciennes Road, Edinburgh, UK

³ Department of Clinical and Surgical Services (Anaesthetics, Pain Management and Intensive Care), Ward 20 ICU, Western General Hospital, Edinburgh, EH4 2XU, UK

⁴ University of Edinburgh, Edinburgh, UK

⁵ Clinical Sciences and Community Health, Medical Statistics Department, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK

⁶ Institute of Paediatrics, Kuala Lumpur Hospital, Jalan Pahang 50586, Kuala Lumpur, Malaysia

E-mail: Robert.Minns@ed.ac.uk

Received 30 October 2002

Published 21 January 2003

Online at stacks.iop.org/PM/24/201

Abstract

A prospective observational study was undertaken to examine time series ICU data of pressure variables (mean arterial pressure (MAP), intracranial pressure (ICP) and cerebral perfusion pressure (CPP)) and relate their variability (SD) to outcome, together with simple graphical displays which could be useful at the ICU bedside.

Forty-three children (aged <1–15 years) were admitted to the intensive care unit for Regional Neurosurgical Service, Edinburgh, following traumatic brain injury (TBI). The standard deviations from 221 291 validated pressure data measurements (representing three variables) were calculated for the duration of ICP monitoring (and in 48 h epochs from the time of injury). Data were displayed on polygraphs, and several well-defined ‘patterns’ were described.

The standard deviations of MAP, ICP and CPP for the total duration of monitoring were found to be significantly related to survival ($p = 0.003$, <0.001 and 0.005 , respectively), while the SD of ICP alone was strongly related to global recovery ($p = 0.008$) in the first 48 h post-injury. Patterns in 104 epochs (each of 48 h) were identified. Ninety-two were of the *type I* (MAP > CPP > ICP) pattern and 12 were of the *non-type I* pattern. Glasgow Outcome Scale scores at 12 months were significantly related to the dichotomized pattern type (Fisher’s exact test $p < 0.001$ for both alive versus

⁷ Author to whom correspondence should be addressed. Department of Child Life and Health, 20 Sylvan Place, Edinburgh, Edinburgh, EH9 1UW, UK.

⁸ Formerly at Royal Hospital for Sick Children, 9 Sciennes Road, Edinburgh, UK.

dead and independent versus dependent outcomes). Only one patient with type I pattern died in this series.

While variability of ICP during the first 48 h post-injury is predictive of the outcome, the pattern behaviour of three pressure signals gives useful outcome prediction information throughout monitoring. These displays may help interpret some of the plethora of data produced at the bedside.

Keywords: children, head injury, intracranial pressure, cerebral perfusion pressure, variability, pattern recognition

1. Introduction and background

Modern intensive care monitoring systems allow displays of many simultaneous biosignals, which generate large quantities of raw data for later off-line analysis. Various summary measures are required to condense the data for analysis, the results of which drive changes towards improving treatments.

The pressure signals of intracranial and cerebral perfusion pressure have generated a great deal of interest, but despite the vast quantity of literature available, there have been relatively few describing their *variability* after traumatic brain injury. Early methods using data from paper recordings (Griffith and Becker 1979, Sklar *et al* 1980, Gaab *et al* 1986) were made, but were hindered by the many technical difficulties, including lengthy traces, chart speed variations, and a lack of objectivity in the quantification and analysis of the data. Computerized monitoring is now the established norm, but there have been few attempts to re-examine these early findings.

Clinical observations of the variability of intracranial pressure, mean arterial pressure and cerebral perfusion pressure (as measured by the standard deviation) led to the hypothesis that the variability of the pressure signals recorded over time after head injury in children may be important in relation to outcome.

This study seeks to view time series data (artefact-excluded) over extended time periods, to investigate the significance of the observed variability and to identify other practical but simple visual displays available at the bedside.

2. Materials and methods

A group of 43 children aged 15 years or younger were identified, with recorded time series data from intensive care monitors, after admission with traumatic head injury. They were included in this study if they fulfilled the following criteria: (a) there was a post-resuscitation pre-intubation Glasgow Coma Score (Teasdale and Jennett 1974) sumscore (GCSs) of 12 or less (the Pediatric Glasgow Coma Score (Reilly *et al* 1988) was used for children 5 years and under) or the GCSs was >12 in association with an Injury Severity Score (Baker and O'Neill 1976) (ISS) of 16 or more, (b) there were clinical indications for monitoring the patients in the intensive care unit and (c) there was a computerized data collection (CDC) system available for use within 24 h of injury.

Clinical notes and nurses' ICU charts were reviewed. Admission details were retrieved, and data including cause and nature of injury, age, post-resuscitation Glasgow Coma Score,

pupil responses, x-ray and computerized tomography (CT), operative and treatment details were extracted.

Children were excluded from the study if they had sustained a previous head injury. Ethical approval for this study was granted through the local ethics committee as part of a larger study of secondary insults, and assent obtained from parent or guardian.

2.1. General management

The management of these children was carried out by experienced neurosurgical, anaesthetic and nursing staff in a specialist unit. The indications for mechanical ventilation and ICP monitoring were: (i) following evacuation of an intracranial haematoma where the patients' GCSs was ≤ 8 ; (ii) where there was a diffuse cerebral injury or GCS ≤ 6 ; (iii) where there was CT or operative evidence of brain swelling; and (iv) where other injuries dictated the need for ventilation. Other monitoring (including arterial pressure, temperature, oxygen saturation and heart rate) was employed as clinically indicated. The ICP was recorded by a Camino catheter or fluid-filled pressure transducer system which was optimally damped using an accudynamic adjustable damping device (Abbott Critical Care System). All monitors were calibrated at least once per day. The management protocol for initiating treatment was a CPP of ≤ 50 mmHg and intracranial pressure (ICP) of ≥ 15 mmHg for children aged <1 –13 years, and for those aged 14–15 years, ≤ 60 and ≥ 20 mmHg respectively. The management protocol was designed to maintain the CPP. If the MAP was low this may have required plasma protein solution (PPS) or noradrenaline. If the MAP was satisfactory but ICP elevated above 20 mmHg, then (1) CO_2 was lowered to 4 kPa; (2) mannitol treatment was instigated (mannitol plus frusemide plus plasma expander) and (3) if ICP remained above 20 mmHg, thiopentone infusion was started. These represent the essentials of an extensive protocol (Miller *et al* 1992) which was set prior to, and maintained for the duration of this study.

2.2. Data acquisition

Data for each patient were collected every minute until clinical monitoring ceased, then manually validated, and any artefact or unreliable data excluded. However, potential iatrogenic derangements, occurring during procedures such as physiotherapy, suction or handbagging, were retained. For this study of pressure signals (ICP, MAP and CPP) data used were restricted to the *duration* of the ICP monitoring which was continued as long as required for clinical management of raised intracranial pressure. From quantitative statistics the mean, range and standard deviation were calculated for each of these parameters, *for each patient*. In addition, the data were divided into 48 h epochs, taken from the time of injury, and the same descriptors recorded for each epoch. The first epoch, taken from the time of injury, always had less than 48 h of data, as this epoch necessarily included the transfer time, admission time and 'set-up' time. Similarly, the last epoch for each child could be less than 48 h, as this depended on a clinical decision as to when the ICP monitor was removed.

For each patient *time series plots* were prepared for each epoch, where the three pressure signals were displayed using the same vertical axis of mmHg. Each 48 h epoch was then assigned a 'pattern type' by consensus among three researchers.

2.3. Outcome

An experienced researcher without access to the physiological data assigned a modified Glasgow Outcome Scale score suitable for children (Adelson *et al* 1997) at 12 months post-injury, after a questionnaire was sent to the parents, and the child's follow-up records were

Table 1. Demographic data for 43 children with ICP monitoring.

Sex	Males	27			
	Females	16			
Cause	Vehicle/bicycle	13			
	Pedestrian	22			
	Falls	4			
	Other	4			
Grade	Severe (GCS ≤ 8)	32			
	Moderate (GCS 9–12)	9			
	Minor (GCS 13–15 with ISS ≥ 16)	2			
Pupils	Some reaction	36			
	No reaction	5			
	Not testable	2			
CT	Focal	24			
	Diffuse	15			
	Less-severe injuries	4			
Outcome at 12 months by grade on admission					
		Severe	Moderate	Mild	Total
	Dead (GOS 1)	8	1	–	9
	Vegetative (GOS 2)	–	–	–	–
	Severe disability (GOS 3)	2	–	–	2
	Moderate disability (GOS 4)	6	3	–	9
	Good recovery (GOS 5)	16	5	2	23
	Total	32	9	2	43

scrutinized. Outcome was dichotomized into independent versus non-recovery (i.e., good recovery and moderate disability (GOS 4–5) versus severe disability, vegetative state or death (GOS 1–3)), and also survival versus death.

3. Analysis

Microsoft® Excel 97 and SPSS® for Windows (Release 9.0.0) packages were used to analyse the data. Mann–Whitney U-tests were used, with the significance level of $p < 0.01$ to ensure non-chance relationships, to test for differences between the outcome categories.

4. Results

The demographic features of the group of 43 children, including age, sex and outcome at 12 months post-injury, are given in table 1.

The duration of ICP monitoring time ranged from 97 to 17 778 min (mean = 6319; median = 3446), with equivalent times for both MAP and CPP, and from the three variables a total of 221 291 usable data points were available for analysis. A total of 104 48 h epochs were identified, the maximum for any individual patient being six epochs (or 12 days). Epochs 1 through 6 had data from 42, 28, 19, 10, 4 and 1 patients, respectively. The mean number of epochs per patient was 2.4. In one case, for technical reasons, computer data were only available from epoch 2 onwards.

Table 2. Significance values of tests for standard deviations and means for both outcome comparisons: alive versus dead and recovered versus not recovered at 12 months post-injury, for epochs 1 to 3, and for total duration of ICP monitoring time.

		Alive versus dead (<i>p</i> -value)	Independent versus not recovered (<i>p</i> -value)
1st epoch (injury to 48 h)			
Mann–Whitney U-test (42)			
ICP	Mean	<0.001*	0.001*
	SD	0.011	0.008*
MAP	Mean	0.053	0.021
	SD	0.193	0.191
CPP	Mean	0.049	0.380
	SD	0.716	0.275
2nd epoch (48–96 h post-injury)			
Mann–Whitney U-test (28)			
ICP	Mean	<0.001*	0.006*
	SD	0.365	1.000
MAP	Mean	0.806	0.709
	SD	0.682	0.784
CPP	Mean	<0.001*	0.006*
	SD	0.978	0.901
3rd epoch (96–144 h post-injury)			
Mann–Whitney U-test (17)			
ICP	Mean	0.006*	0.279
	SD	0.953	0.879
MAP	Mean	0.300	0.442
	SD	0.953	0.721
CPP	Mean	0.244	0.646
	SD	0.859	0.959
Total duration of ICP monitoring			
Mann–Whitney U-test (43)			
ICP	Mean	<0.001*	<0.001*
	SD	<0.001*	<0.001*
MAP	Mean	0.067	0.036
	SD	0.003*	0.017
CPP	Mean	<0.001*	0.003
	SD	0.005	0.021

* Significant at $p < 0.01$.

4.1. Variability

Table 2 reports results for the first three consecutive epochs (admission to 48 h post-injury (epoch 1); 48–96 h (epoch 2) and 96–144 h post-injury (epoch 3)) and for the total duration of acute monitoring, i.e., ICP monitoring time. There were insufficient cases for the analysis of variability in epochs 4 to 6.

The *variability* of ICP, MAP and CPP (as measured by the standard deviation) over the whole time that the patients had an ICP monitor in place, was significantly greater in those who died compared to survivors. Variability of ICP was also significantly greater in those who did not recover compared to those who recovered. Of the three time epochs considered, only the first (i.e., injury to 48 h) showed any relationship between variability and outcome, and then only for ICP. The variability was unrelated to whether on imaging the injury was diffuse or focal.

However, *mean* ICP was significantly related to survival in each of the three time epochs and over the total monitored duration (ICP time), and also to independent recovery (compared to non-recovery) in epochs 1 and 2, and for the total duration. Mean CPP appeared to be important during the second epoch, i.e. between 48 and 96 h post-injury, and also for the total duration of monitoring.

4.2. Patterns

When three variables are displayed on the same graph using a common vertical axis, there are necessarily six permutations, in terms of the order of magnitude in which the variables could theoretically be presented. However, because of the mathematical relationship, $CPP = MAP - ICP$, only three physiologically ordered combinations are possible.

4.3. Descriptions of patterns (see figure 1)

- *Type I*—where MAP was greater than CPP, which was greater than ICP. This would be considered the ‘normal’ and desirable relationship between the variables.
- *Type II*—where MAP was greater than ICP, which was greater than CPP. The ICP is so high that there is insufficient compensation in systemic pressure to maintain adequate CPP.
- *Type III*—where ICP was greater than MAP, which was greater than CPP. With grossly elevated ICP, there is total failure of compensation by systemic pressure and CPP is absent.
- *Type II/I cross-over*—where MAP remains greatest, but CPP changes from being lower than ICP to greater than ICP (i.e., an improving pattern, and is actually a changing pattern from type II to type I).
- *Type I/II cross-over*—where MAP was greatest, but CPP changes from being greater than ICP to less than ICP (i.e. a deteriorating pattern, or changing from type I to type II).
- *Type I/II/III cross-over*—where the three variables MAP, ICP and CPP repeatedly change their relative positions within the epoch, i.e. fluctuating between types I, II and III, although the sequence generally began as a type I.

4.3.1. Patterns in relation to outcome scores. One hundred and four epochs (each of 48 h) were identified from the data of 43 patients and each epoch was classified according to type (figure 2). Type I was the dominant pattern seen in 92 (88.5%) of the 104 epochs and was seen during all 48 h intervals following injury. Of the 34 patients who only displayed type I throughout, only one died, compared to eight deaths in the group of nine patients with non-type I patterns (Fisher’s exact test, $p < 0.001$). A small number of patients (3) had no type I patterns at any time. There were three epochs of type II ($MAP > ICP > CPP$), and one epoch of type III ($ICP > MAP > CPP$). Eight were ‘cross-over’ changing patterns, one of which (in one patient) was an improving cross-over pattern (i.e. type II/I, where the patient recovered well). All patients with other cross-over patterns, or patterns of type II or III died. We found no instances of type III reverting to type I.

4.3.2. Patterns in relation to fatal cases. Nine children died, contributing a total of 20 epochs and nine of these epochs were described as type I. Four patients who died *only* displayed non-type I patterns and at no time had a type I pattern. Of the remaining five patients who died, four had episodes of type I (nine epochs) that subsequently changed to cross-over type I/II or I/II/III. No patient survived if they displayed *at any time epoch* any of the following patterns: type II, type III, cross-over type I/II or type I/II/III.

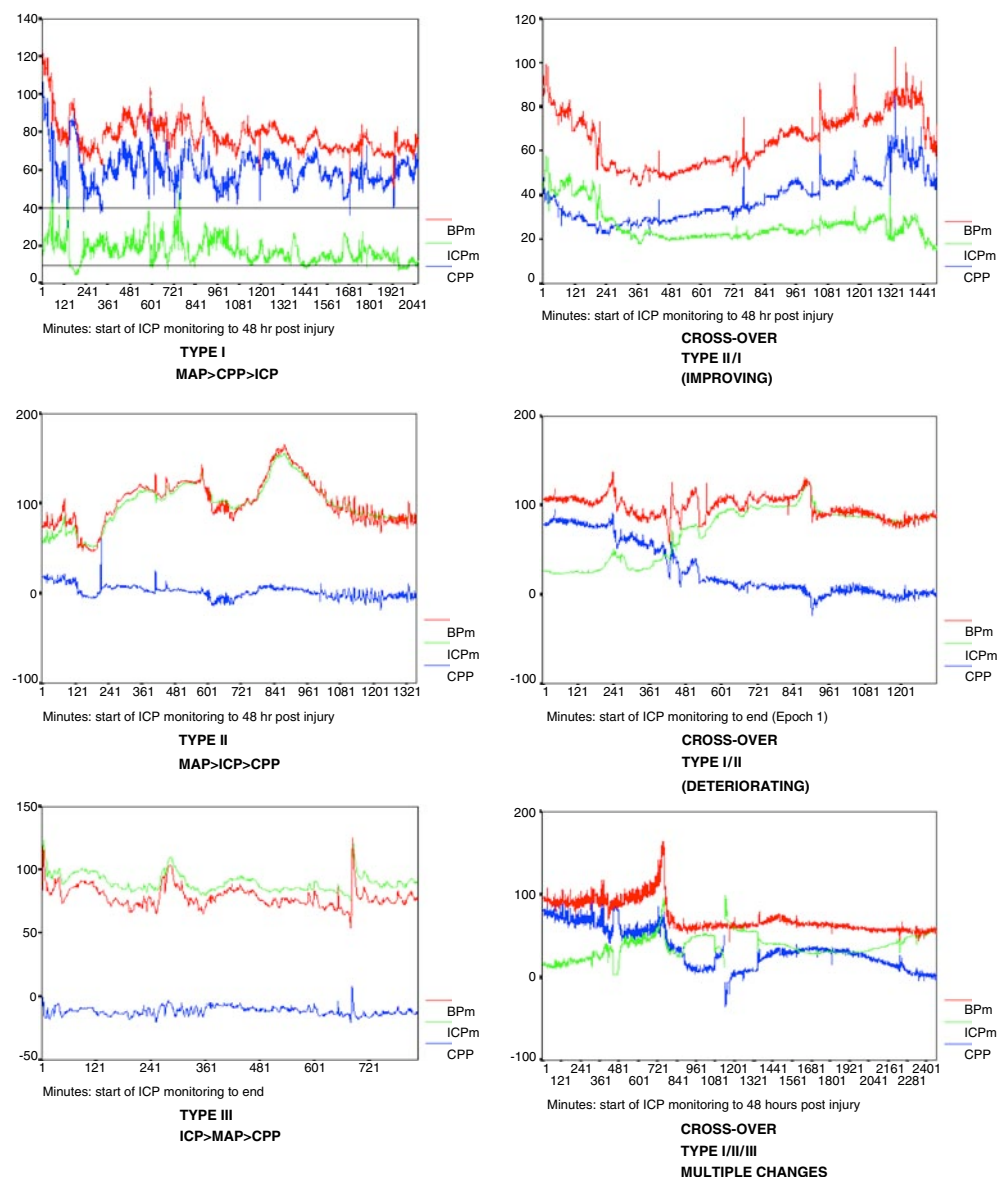


Figure 1. Illustrations of all pattern types identified. MAP displayed in red, mean ICP shown in green and mean CPP shown in blue. Vertical axis in mmHg; horizontal axis in minutes, with tickmarks every 120 min.

The final non-surviving patient had just one epoch of data available which was of the type I pattern. Unfortunately, data were lost for the subsequent epochs, but he eventually died after all monitoring was withdrawn, some 2 days later.

5. Discussion

Clinicians are required to interpret and respond to a great number of increasingly complex clinical parameters displayed on new electronic medical devices, the function of which is to

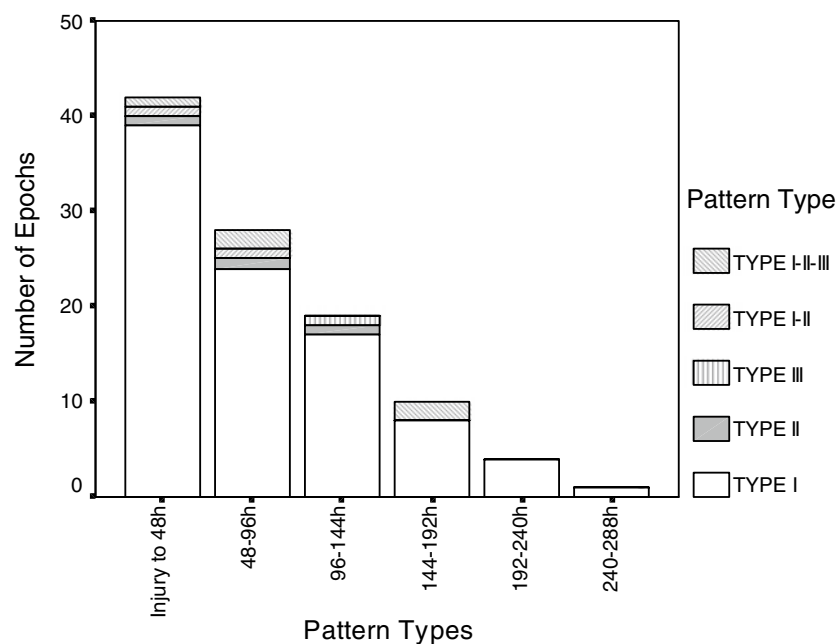


Figure 2. Distribution of 104 pattern types from 43 patients (not mutually exclusive) occurring in epochs 1–6. Each epoch is of 48 h duration, commencing at time of injury. All those displaying types II, III, I-II or I-II-III died.

alert clinicians to significant changes or trends in the patient's condition. With potential data overload, there is a need to *summarize* data for meaningful interpretation.

One way is for nurses to spend valuable time transcribing data displayed on the ITU monitors, onto specially designed charts, at specific time points, e.g. hourly, creating a snapshot in time. This provides a permanent copy of patient information and condenses much of the information into understandable and manageable formats. This has the advantage of being able to view trends over longer time periods than as seen on the monitor screen. The disadvantage is that almost all of the data which have become available with this new technology have been excluded. Most ICP research still uses these hourly transcribed data.

Because on-line data produce lengthy files we conducted our analysis in arbitrary 48 h epochs as well as for the total time. Other researchers have discussed trends over different time points such as (i) overall time taken from commencement of monitoring (Chambers *et al* 2000, Jones *et al* 1994) which gives no indication of time between injury and arrival in ICU, (ii) data truncated to particular time duration of 24, 48 and 72 h from point of impact (Signorini *et al* 1999), or (iii) averaged data over 24 h (Marmarou *et al* 1991b) or 12 h periods (Stocchetti *et al* 1999).

All of the above have disadvantages because the duration of ICP monitoring continues to be dictated by the patient's clinical state. Monitoring may be removed early in those who recover quickly or after physiological stability is achieved. Cortbus (Cortbus *et al* 1994), describing the 'stages' in the pathophysiology of TBI, found that the majority of hypotensive insults occurred within the first 24 h, and ICP derangements clustered around days 5 and 6, although insults were detected up to 12 days. Therefore, by choosing 48 h as our standard epoch, we compromised between a duration long enough to show variations (the cross-over patterns generally take more than 12 h to develop), but not so long that there were overwhelming

Table 3. Methods of analysis of pressure signals.

-
1. ICP stratified (in 10 mmHg bands) from ICP paper traces (Miller *et al* 1977); or raised ICP over 20 mmHg ICP (Miller *et al* 1981)
 2. Digitally averaged ICP, recorded by nurse at the end of each hour (i.e. end hour readings used as estimate of mean ICP), also variance using end hour data (Marmarou *et al* 1991a)
 3. Digital data collected every minute from bedside monitors to use cumulated durations of 'Insults' at three levels of pre-set thresholds (Jones *et al* 1994)
 4. Digital recording (sampled at a rate of 20 Hz), averaged every 12 h, where the highest (ICP) and lowest (CPP) of these values were summarized as 'mean worst' ICP and CPP: the mean for all these 12 h intervals was further summarized as 'mean average' for ICP and CPP (Stocchetti *et al* 1999)
 5. Decision-tree induction from time series (minutely) and demographic data (McQuatt *et al* 1999)
 6. Percentage of total monitored time above ICP (or below (MAP, CPP)) specific thresholds (electronically sampled every 2 min) (Chambers *et al* 2000)
 7. Percent of time CPP in ranges 60–70, 50–60 and <50 mmHg (Jackson *et al* 2000)
 8. Mean CPP, MAP and ICP (using at least hourly data points, from ICU flow sheet for first 48 h), and reduced to single measure of mean and SD for the first 48 h) (Downard *et al* 2000)
 9. Maximum value of 2 min rolling averages of ICP readings calculated over each 1 h period (or minimum value for CPP). ROC curves to attempt to identify thresholds to predict outcome (Chambers *et al* 2001)
 10. Pattern recognition using Bayesian neural networks (Howells 2002)
 11. Standard deviations of ICP, MAP and CPP from digital minutely ICU data in 48 h time frame, and graphical displays (Jones *et al*, this paper)
-

amounts of data to handle. Epochs were well represented with patient deaths occurring in each epoch.

We have not recorded the presence of abnormal ICP wave forms (e.g. plateau, B waves etc) which may influence the standard deviation, but their effect would be relatively minimal when compared to all ICP data points over a 48 h epoch. Additionally, data prior to the last 4 h before death were excluded. We have only considered ICP and BP for analyses here as other monitored physiology (temperature, oxygen saturation and pulse) is not significantly related to the outcome in this dataset (Jones *et al* 2002).

Many methods of analysing physiological data have been described, and these are summarized in table 3. Each of these methods found a relationship with outcome, suggesting that there is no universally accepted standard analysis. We describe a further simple method to aid interpretation of the plethora of data at the bedside.

5.1. Variability

While the mean values of ICP and CPP were highly significant in most of the epochs in relation to outcome as in other studies, the standard deviation of ICP was the only significant predictor (at $\alpha = 0.01$) of non-recovery, and only then in the first 48 h from injury.

Signorini and colleagues examined the 'insults added to injury' at 24, 48 and 72 h post-injury in a population of head-injured adults (Signorini *et al* 1999) and concluded that raised ICP, *however summarized*, was independently predictive of mortality at each of these times. Our findings add a further simple summary measure at a stringent 1% significance level, at a clinically useful time (48 h after injury) which is predictive of morbidity.

A different picture was seen, however, when considering the three pressure signals over the *total* duration of 'acute' monitoring time (i.e. time that ICP monitoring was *in situ*). The variability of all three traces was highly significant in those who died, and the variability of ICP was significantly related to outcome, reflecting the cumulated changes over time. *Practically*, however, the standard deviation (i.e. the variability) over the *whole* trace (if more than 48 h) is not prognostically useful during the acute care of these children, as the variability only becomes significant in retrospect (when it is already evident that the child has survived, or has died).

Marmarou (Traumatic Coma Data Bank group) (Marmarou *et al* 1991a) used ICP 'variance' (standard deviation squared), which was considered to be an indirect measure of the pressure stability, but found that it was only poorly related to outcome. This may be because only 'end hour' readings were used which were considered to be an average estimate of the whole hour, giving only 24 data points per day, compared to our 1440 data points per day for each variable. Clearly, the fewer the data points used, the less variability there is to measure. Gaab (Gaab *et al* 1986) studied a group of 16 head injury patients (including both adults and children) and found that the mean ICP ranged from 20 to 50 mmHg, and the standard deviation ranged from 4 to 21 mmHg: both parameters showed less variability than found in our study of 43 children (mean range 3–99 mmHg; SD range 2–32 mmHg) after artefactual data were removed.

5.2. Pattern types

By categorizing the patterns in this way using the 'order of magnitude', there was ready agreement between the researchers as to the pattern type.

All patients who exhibited patterns of type II or III died, as did all with multiple cross-over (type I/II/III) or simple cross-over (type I/II) patterns. However, the patient with type II/I (changing from a 'poor' to 'better' type of pattern) survived, indicating a response to treatment.

By plotting these three variables on the same vertical scale, patterns emerge that are predictive of survival or death. This would be a simple and useful addition to the ICU nursing charts, where generally numerical values for ICP and CPP are recorded on a separate section of the chart, thus obscuring the information.

However, it was noted that it would be possible to further subdivide type I patterns depending on the *amount* of variability seen in the ICP trace, for example, by identifying those with only 10 mmHg within-trace variation, or 20, 30, 40 or >40 mmHg within-trace variability, or combinations of these values. We were unable to relate these sub-group patterns to the outcome in this sample group of patients as examples were found in all outcome groups.

By viewing these patterns over 48 h time periods, it appeared in some cases as if there was a 4-hourly rhythmicity evident, but on close inspection these were 4 h treatment-related events and not an inherent hydro- or haemo-dynamic pulse.

6. Conclusion

The standard deviation (or variability) of ICP signals frequently recorded during ICU care of paediatric TBI patients gives useful predictive information about outcome during the first 48 h after injury.

The 'time series patterns' generated from traces of three variables (ICP, MAP and CPP) when plotted on the same vertical axis, show clearly which patients are likely to survive or die, and these are useful throughout the whole acute monitoring time. If these values were

plotted on standard ICU nursing charts, they could form a visually useful, simple adjunct to the other methods already available in the management of TBI in the intensive care unit.

References

- Adelson P D, Clyde B, Kochanek P M, Wisniewski S R, Marion D W and Yonas H 1997 Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report *Pediatr. Neurosurg.* **26** 200–7
- Baker S P and O'Neill B 1976 The injury severity score: an update *J. Trauma* **16** 882–5
- Chambers I R, Treadwell L and Mendelow A D 2000 The cause and incidence of secondary insults in severely head-injured adults and children *Br. J. Neurosurg.* **14** 424–31
- Chambers I R, Treadwell L and Mendelow A D 2001 Determination of threshold levels of cerebral perfusion pressure and intracranial pressure in severe head injury by using receiver-operating characteristic curves: an observational study in 291 patients *J. Neurosurg.* **94** 412–6
- Cortbus F, Jones P A, Miller J D, Piper I R and Tocher J L 1994 Cause, distribution and significance of episodes of reduced cerebral perfusion pressure following head injury *Acta Neurochir.* **130** 117–24
- Downard C, Hulka F, Mullins R J, Piatt J, Chesnut R, Quint P and Mann N C 2000 Relationship of cerebral perfusion pressure and survival in pediatric brain-injured patients *J. Trauma—Injury Infection and Critical Care* **49** 654–8
- Gaab M R, Ungersbock K and Hufenbeck B 1986 Evaluation of ICP by computerized bedside monitoring: methods and clinical significance *Neurol. Res.* **8** 44–52
- Griffith R L and Becker D P 1979 Physiological monitoring of the head injury patient *Adv. Neurol.* **22** 51–72
- Howells T P 2002 Pattern recognition in physiological time series data using Bayesian neural networks *PhD Thesis* University of Edinburgh
- Jackson S, Piper I R, Wagstaff A and Souter M 2000 A study of the effects of using different cerebral perfusion pressure (CPP) thresholds to quantify CPP 'secondary insults' in children *Acta Neurochir. Suppl.* **76** 453–6
- Jones P A, Andrews P J, Midgley S, Anderson S I, Piper I R, Tocher J L, Housley A M, Corrie J A, Slattery J and Dearden N M 1994 Measuring the burden of secondary insults in head-injured patients during intensive care *J. Neurosurg. Anesthesiol.* **6** 4–14
- Jones P A, Andrews P J D, Easton V J and Minns R A 2003 Traumatic brain injury in childhood: intensive care time series data and outcome *Br. J. Neurosurg.* at press
- Marmarou A, Anderson R L, Ward J D, Choi S C, Young H F, Eisenberg H M, Foulkes M A, Marshall L F and Jane J A 1991a Impact of ICP instability and hypotension on outcome in patients with severe head trauma *J. Neurosurg.* **75** (suppl) S59–S66
- Marmarou A, Anderson R L, Ward J D, Choi S C, Young H F, Eisenberg H M, Foulkes M A, Marshall L F and Jane J A 1991b NINDS Traumatic Coma Data Bank: intracranial pressure monitoring methodology *J. Neurosurg.* **75** (suppl) S21–S27
- McQuatt A, Andrews P J, Sleeman D, Corbule V and Jones P A 1999 *The Analysis of Head Injury Data using Decision Tree Techniques* ed W Horn (Berlin: Springer) pp 336–45
- Miller J D, Becker D P, Ward J D, Sullivan H G, Adams W E and Rosner M J 1977 Significance of intracranial hypertension in severe head injury *J. Neurosurg.* **47** 503–16
- Miller J D, Butterworth J F, Gudeman S K, Faulkner J E, Choi S C, Selhorst J B, Harbison J W, Lutz H A, Young H F and Becker D P 1981 Further experience in the management of severe head injury *J. Neurosurg.* **54** 289–99
- Miller J D, Dearden N M, Piper I R and Chan K H 1992 Control of intracranial pressure in patients with severe head injury *J. Neurotrauma* **9** (suppl 1) S317–S326
- Reilly P L, Simpson D A, Sprod R and Thomas L 1988 Assessing the conscious level in infants and young children: a paediatric version of the Glasgow Coma Scale *Childs Nerv. Sys.* **4** 30–3
- Signorini D F, Andrews P J D, Jones P A, Wardlaw J M and Miller J D 1999 Adding insult to injury: the prognostic value of early secondary insults for survival after traumatic brain injury *J. Neurol. Neurosurg. Psychiatry* **66** 26–31
- Sklar F H, Beyer C W Jr and Kemp C W 1980 Physiological features of the pressure–volume function of brain elasticity in man *J. Neurosurg.* **53** 166–72
- Stocchetti N, Rossi S, Buzzi F, Mattioli C, Paparella A and Colombo A 1999 Intracranial hypertension in head injury: management and results *Intensive Care Med.* **25** 371–6
- Teasdale G M and Jennett B 1974 Assessment of coma and impaired consciousness. A practical scale *Lancet* **1** 290–1

PAPER 2 (PUBLISHED)

Lo TYM, McPhillips M, Minns RA, Gibson RJ.

Cerebral atrophy following shaken impact syndrome and other non-accidental head injury (NAHI).

Pediatric Rehabilitation. January – March 2003; 6(1): 47-55.

doi: 10.1080/1363849031000109516

Reproduced with the Publisher's permissions

Cerebral atrophy following shaken impact syndrome and other non-accidental head injury (NAHI)

T. Y. M. LO, M. McPHILLIPS, R. A. MINNS and R. J. GIBSON

Accepted for publication: March 2003

Keywords Non-accidental head injuries, cerebral atrophy, microcephaly

Summary

Purpose of the study: To determine the frequency of cerebral atrophy and microcephaly in a group of children with sequential MRI brain scans after surviving a non-accidental head injury ($n = 16$).

Methods: Serial head circumference measurements (OFC) were extracted and plotted on standard growth charts for each child retrospectively to determine the frequency of secondary microcephaly. Cerebral atrophy was diagnosed and quantified by measurement of the ventricular/cortical ratio on coronal images of the sequential scans.

Results: Acquired microcephaly was found in 15 children (93.8%) over a median follow-up period of 67.93 weeks. There was a significant reduction in the median Z-score for the OFC at the most recent follow-up when compared with that at presentation ($p < 0.001$, Wilcoxon Signed Rank Test). Cerebral atrophy was found to be the cause of the microcephaly in eight of the 15 children and was evident as early as 9 days after presentation.

Conclusion: A large proportion of the cohort (93.8%) develops acquired microcephaly after an inflicted head injury and cerebral atrophy is responsible in half of these cases.

Introduction

'Shaken Baby Syndrome' and other non-accidental head injuries (NAHI) are serious conditions of infancy with an annual incidence of 24.6 per 100 000 children younger than 1 year of age [1]. The prognosis following

NAHI is extremely poor with a high mortality rate of 26–36% [2, 3] and up to 78% of the survivors suffering from long-term disability [4]. The morbidity includes epilepsy, visual loss and blindness, language and cognitive impairment, motor disability and other neurodevelopmental delay [5–10] resulting in a significant burden for the carers. This unfavourable outcome is thought to be the result of extensive brain damage arising from both the primary brain injury and additional secondary pathophysiological insults [11] following the trauma. Interference with brain growth following shaking injury, as measured by head circumference, has been reported to occur in three cases reported by Oliver [12] and four of the 12 patients studied by Bonnier *et al.* [9]. This may theoretically be due to either a generalized slowing of brain growth from a global brain insult or it may result from acquired brain atrophy consequent on the injury. The aim was to investigate the frequency of microcephaly and brain atrophy in a cohort of infants who have had sequential MRI scanning following a shaking/impact injury.

Methods

A retrospective review of the clinical and imaging findings from a cohort of children who had been admitted to the Royal Hospital for Sick Children, Edinburgh, with a suspected inflicted head injury was undertaken. The inclusion criteria included all patients (i) who presented with an acute encephalopathy and had at least two of the following features: retinal haemorrhages, skeletal fractures such as rib fractures or metaphyseal avulsions, a history that is changing or inconsistent with the examination findings, subdural haemorrhage or evidence of other malicious injury such as burns; and (ii) had sequential MRI scanning of the brain following the primary injury (table 1).

Serial measurements of the head circumference (OFC) were extracted from the case notes and plotted

Authors: T. Y. M. Lo, Clinical Research Fellow; R. A. Minns (author for correspondence; e-mail: robert.minns@ed.ac.uk), Consultant Paediatric Neurologist & Academic Head, Department of Paediatric Neurosciences; M. McPhillips, Consultant Paediatric Radiologist; and R. J. Gibson, Consultant Radiologist, Department of Paediatric Radiology, Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, UK.

Table 1 Summary of the clinical features, initial MRI finds, interventions and outcome of the cohort

Patient	Age at presentation	Clinical features	Initial MRI features	Interventions	Outcome
1	5.6 weeks	(1) Linear parietal skull fracture (2) Bilateral retinal haemorrhages (3) Bruise on the right side of the face	(1) Extensive bilateral subdural haematomata	(1) Subdural taps	Alive with age appropriate development
2	10.1 weeks	(1) Bilateral retinal haemorrhages (2) Seizures	(1) Bilateral subdural haematomata	(1) Subdural taps (2) Seizure control	Alive with moderate developmental delay
3	4.0 weeks	(1) Tense fontanelle (2) Sunseting (3) Stridor (4) Occipital swelling (5) Facial bruising (6) Multiple rib fractures (7) Seizures	(1) Moderate ventricular dilatation (2) Cerebellum and tonsillar herniation (3) Right subdural, sub-arachnoid and intraventricular haemorrhages	(1) Ventricular taps (2) Ventriculo-peritoneal shunt	Alive with moderate developmental delay
4	8.9 weeks	(1) Bruising over the right scapula (2) Abrasion on the left anterior chest wall	(1) Bilateral subdural haematomata (2) Bilateral sub-arachnoid haemorrhages (3) Thin subdural blood underneath the tentorium and sub-temporally	(1) Supportive	Alive with age appropriate development
5	4.0 weeks	(1) Large head (2) Bilateral retinal haemorrhages	(1) Bilateral subdural haematomata	(1) Subdural taps (2) Subduroperitoneal shunt	Alive with mild developmental delay
6	5.2 weeks	(1) Diffuse petechiae over upper body and face (2) Linear bruises on both shins and behind the left knee (3) Tense fontanelle (4) Seizures (5) Bilateral retinal haemorrhages (6) Multiple rib fractures	(1) Right sub-arachnoid haemorrhage (2) Loss of grey-white differentiation in area supplied by the right middle cerebral artery (3) No evidence of subdural haemorrhage	(1) Manitol (2) Seizure control	Alive with left hemiplegia and severe developmental delay
7	19.1 weeks	(1) Seizure (2) Bradycardia (3) Oxygen desaturation (4) Bruise over the right mandible (5) Bilateral retinal haemorrhages (6) Healing multiple rib fractures (7) Metaphyseal corner fractures of both femora (8) Left parietal skull fracture (9) Large head	(1) Bilateral subdural haematomata (2) Blood in the occipital horns of the lateral ventricles and around the cerebellum (3) Mild dilation of the lateral and third ventricles	(1) Subdural taps (2) Subdural drains (3) Subduroperitoneal shunts	Alive with moderate developmental delay

Cerebral atrophy

8	5.5 weeks	(1) Seizures (2) Left facial bruising	(1) Bilateral subdural haematomata (2) Bilateral temporal and parietal contusion	(1) Seizure control (2) Subdural taps	Alive with severe developmental delay
9	2.4 weeks	(1) Swelling over the right side of head (2) Right parietal skull fracture (3) Metaphyseal fracture of the right femur	(1) Right subgaleal haematoma Right subdural haematoma	(1) Supportive	Alive with age appropriate development
10	6.0 weeks	(1) Large head (2) Bilateral retinal haemorrhages	(1) Bilateral subdural haematomata	(1) Supportive	Alive with mild developmental delay
11	7.0 weeks	(1) Tense fontanelle (2) Apnoea (3) Seizures (4) Bilateral retinal haemorrhages	(1) Bilateral subdural haematomata (2) Bilateral subarachnoid haemorrhages	(1) Subdural taps (2) Subdural drains	Alive with moderate developmental delay
12	13.0 weeks	(1) Tense fontanelle (2) Bilateral sub-conjunctival haemorrhages (3) Fluctuating conscious level (4) Bilateral retinal haemorrhages (5) Seizures (6) Metaphyseal corner fracture of the left tibia	(1) Bilateral subdural and sub-arachnoid haemorrhages including posterior fossa	(1) Subdural drains	Alive with moderate developmental delay
13	28.9 weeks	(1) Epitaxis (2) Bruising to the chest, penis, buttocks and spine (3) Bilateral retinal haemorrhages	(1) Enlarged ventricles (2) Increased pericerebral spaces (3) Bilateral subdural haematomata (4) Bilateral sub-arachnoid haemorrhages	(1) Subdural taps	Alive with mild developmental delay
14	3.1 weeks	(1) Seizures (2) Full fontanelle (3) Bilateral retinal haemorrhages	(1) Bilateral subdural haematomata	(1) Seizure control (2) Supportive	Alive with mild developmental delay
15	10.5 weeks	(1) Seizures (2) Cyanosis (3) Hypotension (4) Bruising below the left eye (5) Full fontanelle (6) Bilateral retinal haemorrhages	(1) Bilateral subdural haematomata (2) Left parietal parachymal tear	(1) Subdural taps (2) Subdural drains	Alive with severe developmental delay
16	19.3 weeks	(1) Seizures (2) Bradycardia (3) Bulging fontanelle (4) Petechiae over the right palpebral fissure (5) Bilateral retinal haemorrhages	(1) Right subdural haemotoma (2) Asymmetry of the lateral ventricles	(1) Manitol (2) Subdural drains	Alive with moderate developmental delay

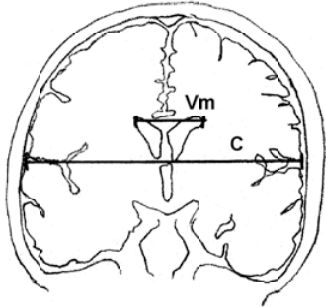


Figure 1 A coronal MRI brain image at the level of Foramen of Monro is used to measure the bifrontal ventricular diameter (Vm) and the maximum cortical diameter (C). The ventricular/cortical (V/C) ratio is then calculated by Vm/C .

on normal growth centile charts for each patient. The frequency of microcephaly, defined as 2 SD below the mean, was determined. The Z-score (the number of standard deviations from the mean) was calculated for the OFC measurement at presentation and at the most recent follow-up.

All the MRI brain images were obtained using a Siemens Magnetom 1.5T Scanner or a Picker Outlook 0.23T Scanner. The timing of the scans after the acute presentation was recorded. Two neuroradiologists reviewed the sequential scans independently and determined the bifrontal ventricular diameter (Vm) and the maximum cortical diameter (C) on the coronal image at the level of Foramen of Monro (figure 1). The ventricular/cortical (V/C) ratio was then calculated. Cerebral atrophy was recognized both by the prominence of the gyri and sulci pattern with increased CSF spaces and was deemed the cause of the microcephaly when the Vm and V/C ratio was increasing.

Statistical analysis using Wilcoxon Signed Rank Test was carried out on the Z-scores of the OFC, the Vm and the V/C ratio on the scans at presentation and the most recent follow-up examination.

Results

Sixteen infants (10 boys and six girls) who fulfilled the study criteria were identified. The median age at presentation was 7 weeks (2.43–28.86 weeks). A total of 143 OFC measurements (a median of eight measurements per patient, range 2–16) were extracted over a median follow-up period of 67.93 weeks (range 24–92) post-injury. There were 52 MRI scans (a median of three MRI scans per patient, range 2–4 scans) obtained over a median follow-up period of 19.86 weeks (range 1.14–134.70 weeks) after presentation.

Fifteen of the 16 infants (93.75%) had evidence of microcephaly at the most recent follow-up. The serial OFC measurements obtained from each of these 15 infants crossed the centiles to more than 2 SD below

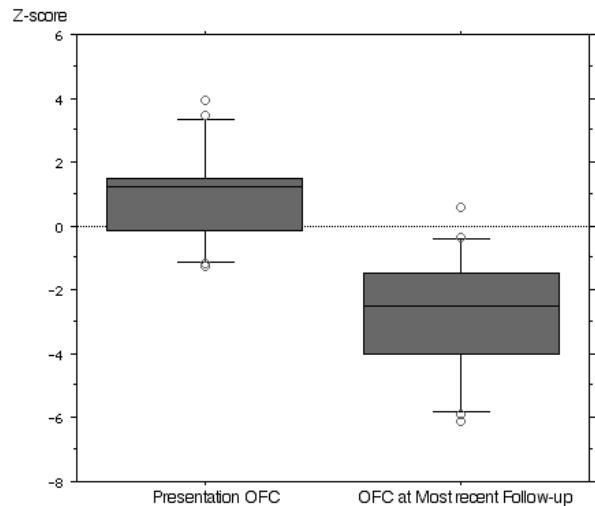


Figure 2 Box plot showing the median Z-score (number of standard deviations from the mean) of the head circumference (OFC) at presentation and at the most recent follow-up. ($p < 0.001$, Wilcoxon Signed Rank Test.)

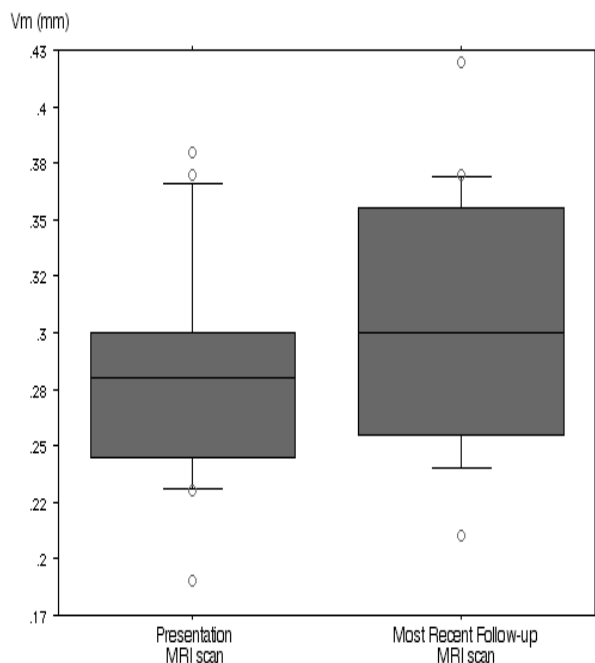


Figure 3 Box plot showing the bifrontal ventricular diameter (Vm) obtained from the MRI scans done at presentation and at most recent follow-up.

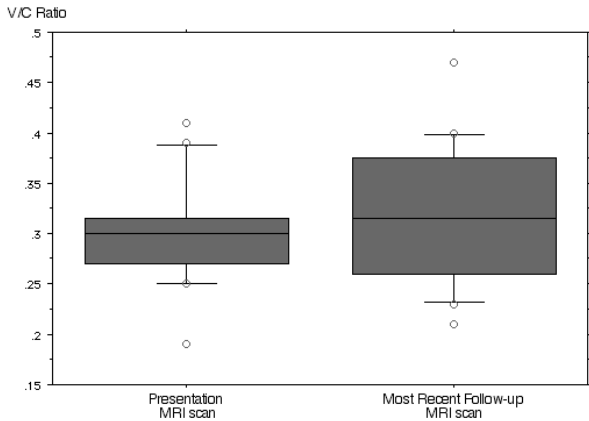


Figure 4 Box plot showing the ventricular/cortical (V/C) ratio obtained at the MRI scans done at presentation and at most recent follow-up.

the mean when plotted on the growth charts for head circumference, indicating a failure to maintain a normal head growth pattern. The median Z-score for the OFC at presentation was +1.23 (−1.25 to +3.92) and at most recent follow-up was −2.51 (−6.11 to +0.58), which was statistically significant ($p < 0.001$) (figure 2). The median time for the OFC to first crossing the third centile was 59.0 weeks from presentation (range 8.0–90.5 weeks).

The median Vm and V/C ratios on the first scan after admission were 28.5 mm (18–52 mm) and 0.30 (0.19–0.41) respectively, while those on the most recent follow-up scan were 33.00 mm (21–53 mm) and 0.32 (0.21–0.47) respectively. The changes in Vm and V/C ratio did not reach statistical significance (figure 3 and 4). The sequential MRI brain scans from eight of the 15 infants (53.3%) with microcephaly showed evidence of progressive increase in Vm and V/C ratio, indicating cerebral atrophy was the cause of the microcephaly in these patients. The earliest recognition of cerebral atrophy on MRI scan in this study was 9 days after presentation.

CASE 1

A 6 week-old girl presented with an acute encephalopathy, bilateral retinal haemorrhages and bilateral subdural haematoma, but without any signs of physical neglect or fractures. MRI brain scans were performed on the day of presentation, and 6, 14 and 60 days after admission (figure 5). The V/C ratio increased with time and the serial OFC measurements declined below the normal growth centiles indicating an acquired microce-

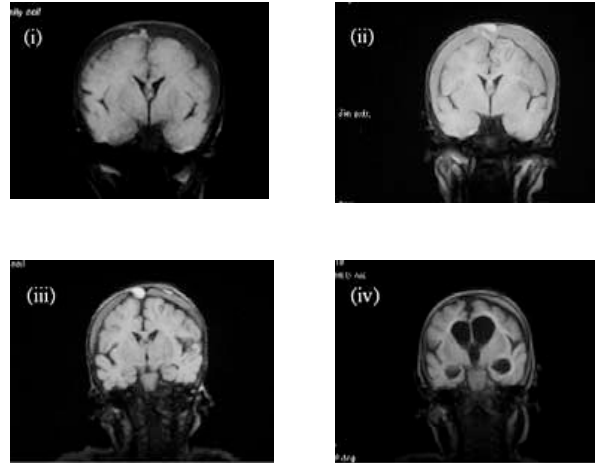


Figure 5 Sequential MRI brain scans showing the development of acquired cerebral atrophy from (i) the day of presentation, (ii) 6 days, (iii) 14 days to (iv) 2 months post-presentation in a 6 week-old girl who presented with acute encephalopathy, bilateral retinal haemorrhages and bilateral subdural haematoma.

phaly. The most recent OFC measurement was 4.66 SD below the mean (figure 6).

CASE 2

This 10 week-old boy presented with an acute encephalopathy, bruising below the left eye, bilateral retinal haemorrhages and bilateral subdural haematoma with hypovolaemic shock requiring mechanical ventilation and inotropic support. The first MRI brain scan was performed 3 days after admission. Evidence of cerebral atrophy was first observed from the scans 12 days after presentation. The most recent follow-up scan, 1.07 years after presentation, showed marked cerebral atrophy (figure 7). Serial OFC measurement demonstrated the evolution of severe microcephaly with an OFC at the most recent follow-up being 6.11 SD below the mean (figure 8).

CASE 3

A 5 week-old boy presented with acute encephalopathy, multiple rib fractures, bilateral retinal haemorrhages and bilateral subdural haematoma with raised intracranial pressure and requiring neurointensive care. MRI brain scans were obtained on the first and ninth days after admission, 9 months and 2 years post-presentation and showed the rapid development of asymmetrical cerebral atrophy (figure 9). Acquired microcephaly was evident from the serial OFC meas-

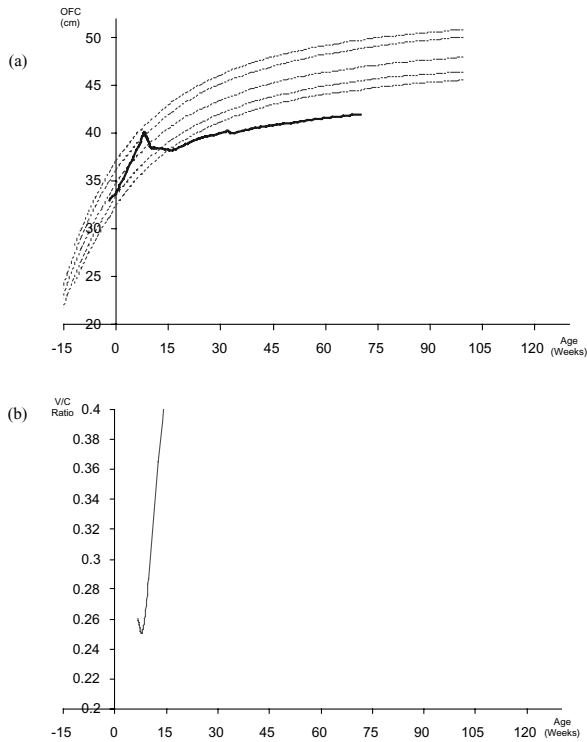


Figure 6 (a) Head circumference (OFC) falling off the normal growth centiles with the latest OFC being 4.66 SD below the mean. (b) Plot showing increased V/C ratio over time.

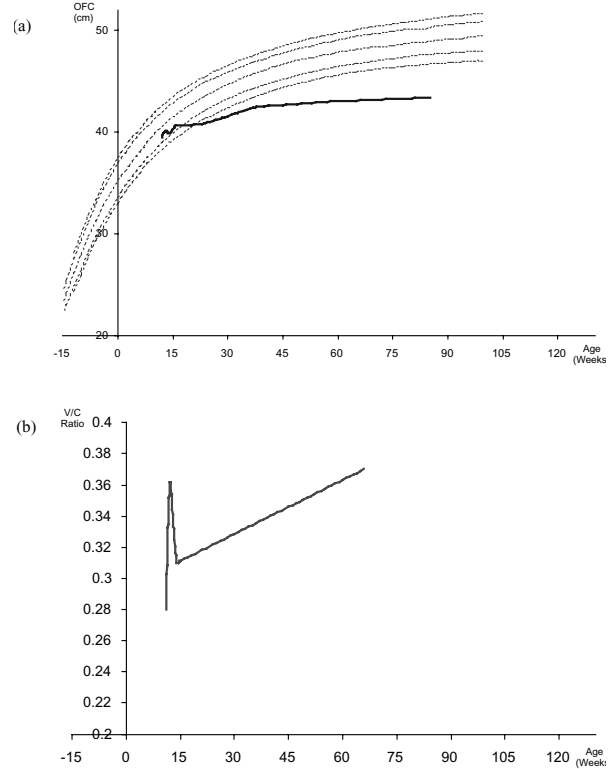


Figure 8 (a) A head circumference growth chart demonstrating failure to maintain a normal head growth pattern with the latest OFC measurement being 6.11 SD below the mean. (b) Increased V/C ratio was shown over time.

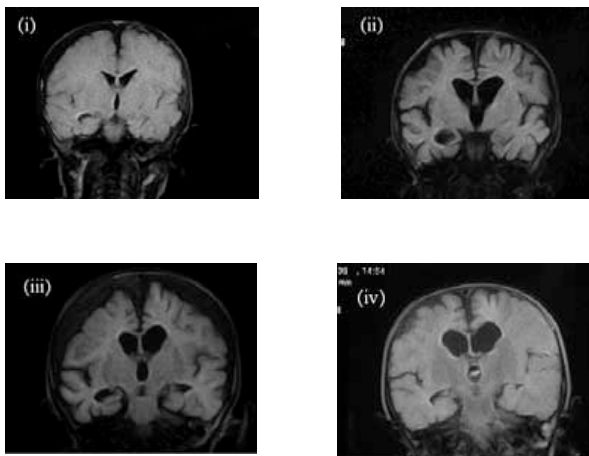


Figure 7 Serial MRI brain scans performed on a 10 week-old boy with bruising below the left eye, acute encephalopathy, bilateral retinal haemorrhages, bilateral haematoma and hypovolaemic shock requiring intensive care treatment at (i) 3 days, (ii) 12 days, (iii) 3.71 weeks and (iv) 1.07 years after presentation showing evolution of cerebral atrophy.

urement with the most recent follow-up OFC being 5.88 SD below the mean (figure 10).

Discussion

This retrospective study of infants surviving a NAHI who additionally had sequential brain MRI scans has demonstrated that over half (53.3%) of the cohort developed cerebral atrophy, with the earliest imaging evidence of atrophy being observed as soon as 9 days after presentation. Cerebral atrophy is well recognized after various brain insults. Jaworski *et al.* [13] reported the presence of cerebral atrophy in 91% of microcephalic children following perinatal or post-natal insults including hypoxic ischaemic encephalopathy, intracranial haemorrhage or meningitis. Adults who underwent sequential CT scanning after sustaining severe brain trauma developed cerebral atrophy in 28% of cases, but this incidence rose to 83% in those patients who had suffered an acute subdural haematoma [14].

Cerebral atrophy

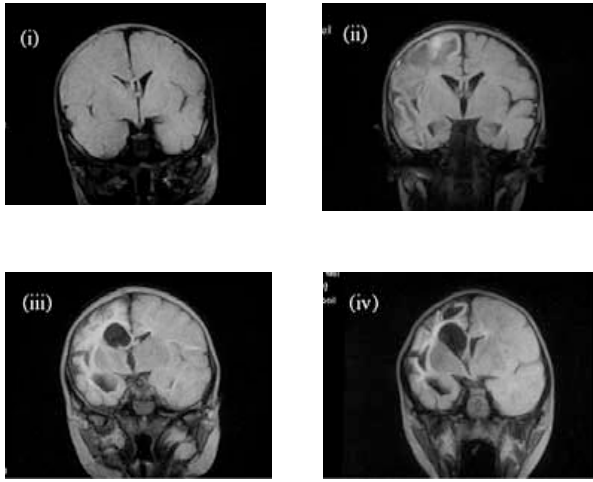


Figure 9 A 5 week-old boy who presented with acute encephalopathy, multiple rib fractures, bilateral retinal haemorrhages and bilateral subdural haematoma with resultant raised intra-cranial pressure requiring neurointensive care had sequential MRI scans done at (i) 1 day, (ii) 11 days, (iii) 9 months and (iv) 2.3 years after presentation. The scans showed the development of asymmetrical cerebral atrophy.

The incidence of cerebral atrophy is less well documented in children following accidental head injury, but Onuma *et al.* [15] reported on five children with severe cerebral atrophy demonstrated on CT scans and all were associated with poor outcome. There is no previous report on the frequency of acquired cerebral atrophy following NAHI.

The mechanisms responsible for inflicted brain injury are largely rotational deceleration forces experienced in the infant's cranium [8, 16] although other additional impact and compression injuries may also be responsible in some cases. Only in high velocity road traffic accidents do children experience similar impact and rotational injuries. Virtually all infants suffering from NAHI have extensive subdural haematoma [17] and the subdural haemorrhage may be very extensive and involve the convexity, interhemispheric, sub-temporal and sub-occipital regions [17]. Beneath the subdural haematoma, in the underlying cerebral parenchyma, in particular the white matter, there is hypoperfusion with ischaemia and infarction, an uncoupling of flow and metabolism, and oedema (cytotoxic compressive and vasogenic) with resultant atrophy in the long term [18]. Cytokine induced gliosis may also contribute to the resultant atrophy. The other imaging evidence of brain injury following shaking with or without impact includes tearing of the bridging veins [8, 16, 17], diffuse axonal injury (petechiae at the grey-white matter junction, corpus callosum and sub-cortical white matter

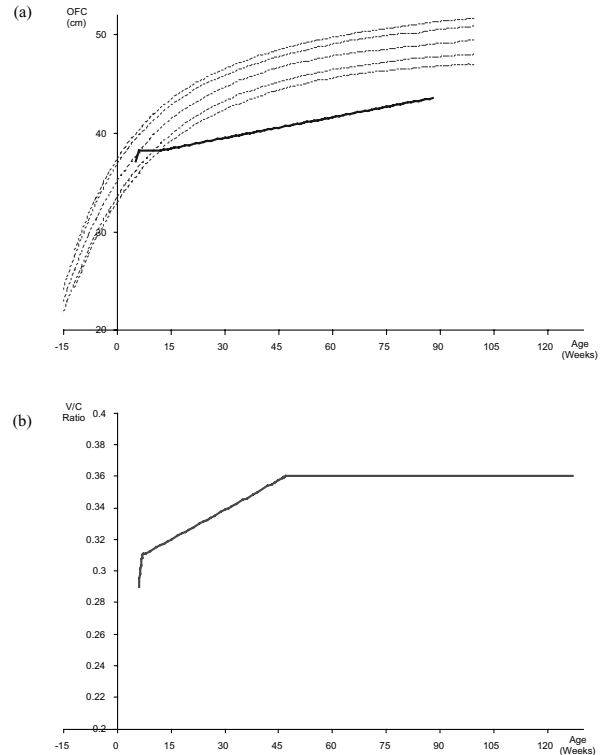


Figure 10 (a) The serial OFC measurements showed a slowing of head growth below the normal with the latest OFC being 5.88 SD below the mean. (b) The V/C ratio increased steadily over time until a plateau was reached.

tears) [19–21], cerebral oedema and hypoxic ischaemic changes. It is believed that this is the first documented report of cerebral atrophy following NAHI in children.

Although radiological description of cerebral atrophy is often subjective and dependent on pattern recognition, one has attempted a more objective assessment by measurements of ventricular dimension and ventricular/cortical ratio allowing for more accuracy and better comparison between sequential scans obtained in the same patient as well as between different patients and allows for the non-linear segmental increase in ventricular volume which occurs with normal growth throughout childhood [22].

Global slowing of brain growth will result in microencephaly with a proportional loss of cortex, sub-cortical grey and white matter, and the Vm and V/C ratio will, therefore, remain constant on sequential scanning. Specific white matter atrophy will result in a disproportional loss of white matter in relation to the sub-cortical grey matter and global atrophy (of both white and grey matter) are determined indirectly by

the increasing V/C ratio on sequential scans in the presence of slowing of head growth.

In this study, the first imaging evidence of cerebral atrophy was seen at 9 days after presentation, earlier than the 2 weeks reported for its appearance after accidental head injury [14, 15]. Barlow *et al.* [17] reported the additional information obtained from acute MRI scans about the nature and extent of the inflicted injuries compared to those provided by CT scans, and it is postulated that follow-up MRI scans will likewise contribute additionally to the knowledge of the evolving intraparenchymal pathology as well as the changing appearance of the sub-temporal and posterior fossa regions during the rehabilitation process.

All of the patients in this study with cerebral atrophy also had microcephaly. Significant global insults to the immature brain will frequently result in secondary microcephaly, which is seen in 17.6–48% of infants at 12 months after perinatal hypoxic ischaemic encephalopathy [23, 24]. Microcephaly is also well recognized to occur after central nervous system infections from cytomegalovirus [25], varicella zoster [26], HIV [27] and other pyogenic meningitis [28]. Oliver [12] described the development of acquired microcephaly in three children following shaking, swinging, hitting or throwing injury in 1979 and Bonnier *et al.* [9] reported four of their 12 abused children surviving an inflicted head injury who developed atrophy with interference of head growth occurring 4 months after the presumed time of injury. Thus, this study has suggested a much higher frequency of NAHI induced microcephaly than previously reported. Furthermore, although the first evidence of cerebral atrophy can be observed soon after presentation in the cohort, the first evidence of microcephaly took much longer to become clinically apparent.

The social circumstances for many of these infants are often complex, making the pre-injury details including head size difficult to obtain. Only five of the 16 infants had records of earlier OFC measurements, all of which were within normal centiles. Four of these children had OFC measurements at follow-up significantly lower than their pre-morbid centiles. The head circumference may be transiently and artificially increased in the acute encephalopathy as a result of scalp oedema, subgaleal haemorrhage and acute subdural haematomata. All but one of the cohort had microcephaly at the most recent follow-up and, more importantly, their serial OFC measurements obtained during the rehabilitation period showed failure to maintain a normal rate of head growth confirming the pathological nature of the pattern.

Nineteen per cent of the cohort had age-appropriate development without any neurological deficits, which was similar to the proportion of symptom-free NAHI survivors reported in the literature [29–34]. Although it is clearly important to study the neuropsychological and neuropsychometric deficits consequent on an inflicted brain injury during infancy, the very young age of the population (median age of 7 weeks at presentation), which also included some ex-preterm babies, and the relatively short period of clinical follow-up (median 67.93 weeks) meant that only significant sequelae could be deduced at the time of out-patient clinic visits from routine developmental examination and recognition of specific neurological deficits such as blindness and epilepsy.

The neurological deficits are very much in evolution and deciding the definitive outcome based on any duration less than the whole of childhood may be misleading, as Bonnier *et al.* [9] have demonstrated in their cohort of 13 infants (mean age of 5.5 months at presentation, who were followed up for a mean of 7 years) that, although an interruption of brain growth was demonstrable at 4 months post-injury, the evolution of long tract signs took 6–12 months, epilepsy took 2 years and behavioural and psychological consequences took between 3–6 years to develop following the initial insult. A detail neuropsychological study on a larger cohort has been completed and will be reported in the near future (Barlow *et al.*, personal communication).

The true incidence of microcephaly and acquired cerebral atrophy following NAHI in childhood remain unknown, but in this study where patients were chosen because of the nature of their injuries together with the availability of their sequential MRI brain scans, it is suggested that microcephaly and cerebral atrophy occur in a very high proportion of infants (93.75% and 53.3%, respectively). This has highlighted the need to anticipate deficits that are likely to evolve as the infants mature particularly in the areas of speech, language and psychomotor development. Development of future management strategies in these areas should be anticipatory and concentrate on preventative measures and early intervention before the appearance of the psychomotor deficits.

References

1. BARLOW, K. and MINNS, R.: Annual incidence of shaken impact syndrome in young children. *Lancet*, **356**: 1571–1572, 2000.
2. KALFF, R., KOCKS, W., POSPIECH, J., *et al.*: Clinical outcome after head injury in children. *Child's Nervous System*, **5**: 156–159, 1989.

Cerebral atrophy

3. GOLDSTEIN, B., KELLY, M., BRUTON, D., *et al.*: Inflicted versus accidental head injury in critically injured children. *Critical Care Medicine*, **21**: 1328–1332, 1993.
4. BARLOW, K., THOMSON, E., MINNS, R. *et al.*: The neurological and neuropsychological outcome of non-accidental head injury. *European Journal of Paediatric Neurology*, **3**: A139 (Abstract), 1999.
5. BARLOW, K., MILNE, S., AITKEN, K., *et al.*: A retrospective epidemiological analysis of non-accidental head injury in children in Scotland over a 15 year period. *Scottish Medical Journal*, **43**: 112–114, 1998.
6. HAVILAND, J. and RUSSELL, R.: Outcome after severe non-accidental head injury. *Archives of Disease in Childhood*, **77**: 504–507, 1997.
7. DUHAIME, A., CHRISTIAN, C., MOSS, E., *et al.*: Long-term outcome in infants with the shaking-impact syndrome. *Pediatric Neurosurgery*, **24**: 292–298, 1996.
8. BROWN, J. and MINNS, R.: Non-accidental head injury, with particular reference to whiplash shaking injury and medico-legal aspects. *Developmental Medicine & Child Neurology*, **35**: 849–869, 1993.
9. BONNIER, C., NASSOGNE, M., EVRARD, P. *et al.*: Outcome and prognosis of whiplash shaken infant syndrome; late consequences after a symptom-free interval. *Developmental Medicine & Child Neurology*, **37**: 943–956, 1995.
10. BARLOW, K., SPOWART, J., MINNS, R. *et al.*: Early posttraumatic seizures in non-accidental head injury: relation to outcome. *Developmental Medicine & Child Neurology*, **42**: 591–594, 2000.
11. BARLOW, K. and MINNS, R.: The relation between intracranial pressure and outcome in non-accidental head injury. *Developmental Medicine & Child Neurology*, **41**: 220–225, 1999.
12. OLIVER, J.: Microcephaly following baby battering and shaking. *British Medical Journal*, **2**: 262–264, 1975.
13. JAWORSKI, M., HERSH, J., DONAT, J., *et al.*: Computed tomography of the head in the evaluation of microcephaly. *Pediatrics*, **78**: 1064–1069, 1986.
14. TOMITA, H., ITO, U., SAITO, J., *et al.*: Cerebral atrophy after severe head injury. *Advances in Neurology*, **52**: 553, 1990.
15. ONUMA, T., SHIMOSEGAWA, Y., KAMEYAMA, M., *et al.*: Clinicopathological investigation of gyral high density on computerized tomography following severe head injury in children. *Journal of Neurosurgery*, **82**: 995–1001, 1995.
16. DUHAIME, A., GENNARELLI, T., THIBAUT, L., *et al.*: The shaken baby syndrome. A clinical, pathological, and biomechanical study. *Journal of Neurosurgery*, **66**: 409–415, 1987.
17. BARLOW, K., GIBSON, R., MCPHILLIPS, M., *et al.*: Magnetic resonance imaging in acute non-accidental head injury. *Acta Paediatrica*, **88**: 734–740, 1999.
18. SHAVER, E., DUHAIME, A., CURTIS, M., *et al.*: Experimental acute subdural hematoma in infant piglets. *Pediatric Neurosurgery*, **25**: 123–129, 1996.
19. GEDDES, J., HACKSHAW, A., VOWLES, G., *et al.*: Neuropathology of inflicted head injury in children. I. Patterns of brain damage. *Brain*, **124**: 1290–1298, 2001.
20. GEDDES, J., VOWLES, G., HACKSHAW, A., *et al.*: Neuropathology of inflicted head injury in children. II. Microscopic brain injury in infants. *Brain*, **124**: 1299–1306, 2001.
21. SHANNON, P., SMITH, C., DECK, J., *et al.*: Axonal injury and the neuropathology of shaken baby syndrome. *Acta Neuropathologica*, **95**: 625–631, 1998.
22. XENOS, C., SGOUROS, S., NATARAJAN, K. *et al.*: Ventricular volume change in childhood. *Journal of Neurosurgery*, **97**: 584–590, 2002.
23. MERCURI, E., RICCI, D., COWAN, F., *et al.*: Head growth in infants with hypoxic-ischemic encephalopathy: correlation with neonatal magnetic resonance imaging. *Pediatrics*, **106**: 235–243, 2000.
24. WOLF, M., WOLF, B., BIJLEVELD, C., *et al.*: Acquired microcephaly after low Apgar score in Zimbabwe. *Journal of Tropical Pediatrics*, **45**: 281–286, 1999.
25. NOYOLA, D., DEMMLER, G., NELSON, C., *et al.*: Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *Journal of Pediatrics*, **138**: 325–331, 2001.
26. SCHEFFER, I., BARAITSER, M., BRETT, E. *et al.*: Severe microcephaly associated with congenital varicella infection. *Developmental Medicine & Child Neurology*, **33**: 916–920, 1991.
27. KOZLOWSKI, P., BRUDKOWSKA, J., KRASZPULSKI, M., *et al.*: Microencephaly in children congenitally infected with human immunodeficiency virus—a gross-anatomical morphometric study. *Acta Neuropathologica*, **93**: 136–145, 1997.
28. HARVEY, D., HOLT, D., BEDFORD, H. *et al.*: Bacterial meningitis in the newborn: a prospective study of mortality and morbidity. *Seminars in Perinatology*, **23**: 218–225, 1999.
29. LUDWIG, S. and WARMAN, M.: Shaken baby syndrome: a review of 20 cases. *Annals of Emergency Medicine*, **13**: 104–107, 1984.
30. FRANK, Y., ZIMMERMAN, R., LEEDS, N. *et al.*: Neurological manifestations in abused children who have been shaken. *Developmental Medicine & Child Neurology*, **27**: 312–316, 1985.
31. SINAI, S. and BALL, M.: Head trauma due to child abuse: serial computerized tomography in diagnosis and management. *Southern Medical Journal*, **80**: 1505–1512, 1987.
32. ZEPP, F., BRUHL, K., ZIMMER, B., *et al.*: Battered child syndrome: cerebral ultrasound and CT findings after vigorous shaking. *Neuropediatrics*, **23**: 188–191, 1992.
33. WILKINSON, W., HAN, D., RAPPLEY, M., *et al.*: Retinal hemorrhage predicts neurologic injury in the shaken baby syndrome. *Archives of Ophthalmology*, **107**: 1472–1474, 1989.
34. BRENNER, S. and FISCHER, H.: The shaken baby syndrome. *Journal of Neurosurgery*, **68**: 660–661, 1988.

Copyright © 2003 EBSCO Publishing

PAPER 3 (PUBLISHED)

**Jones PA, Chambers IR, Lo TYM, Andrews PJD, Chaudhry W, Clark A,
Croft J, Forsyth R, Fulton B, Mendelow AD, Wilson G, Minns RA.**

**Quantification of secondary CPP insult severity in paediatric head
injured patients using a pressure time index.**

***Acta Neurochirurgica*. 2005; 95(Supplement): 29 – 32.**

Reproduced with the Publisher's Permissions.

Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure-time index

P. A. Jones¹, I. R. Chambers², T. Y. M. Lo¹, P. J. D. Andrews³, W. Chaudhry⁴, A. Clark², J. Croft⁵, R. Forsyth⁴, B. Fulton⁶, A. D. Mendelow⁵, G. Wilson⁵, and R. A. Minns¹

¹Child Life and Health, University of Edinburgh, Edinburgh, UK

²Regional Medical Physics Department, Newcastle General Hospital, Newcastle upon Tyne, UK

³Department of Anaesthetics, University of Edinburgh, Edinburgh, UK

⁴Department of Paediatrics, Newcastle General Hospital, Newcastle upon Tyne, UK

⁵Department of Neurosurgery, Newcastle General Hospital, Newcastle upon Tyne, UK

⁶Department of Anaesthetics, Newcastle General Hospital, Newcastle upon Tyne, UK

Summary

This paper describes and validates a new *Cumulative Pressure-Time Index* (CPT) which takes into account both duration and degree of cerebral perfusion pressure (CPP) derangement and determines critical thresholds for CPP, in a paediatric head injury dataset.

Sixty-six head-injured children, with invasive minute-to-minute intracranial pressure (ICP) and blood pressure monitoring, had their pre-set CPP derangement episodes (outside the normal range) identified in three childhood age-bands (2–6, 7–10, and 11–16 years) and global outcome assessed at six months post injury.

The new cumulative pressure-time index more accurately predicted outcome than previously used summary measures and by varying the threshold CPP values, it was found that these physiological threshold values (≤ 48 , ≤ 52 and ≤ 56 mmHg for 2–6, 7–10, and 11–16 years respectively) best predicted brain insult in terms of subsequent mortality and morbidity.

Keywords: Paediatric head injury; cumulative pressure-time index; critical CPP thresholds; outcome.

Introduction

Cerebral perfusion pressure (CPP) has been shown to be the best predictor of outcome in both adult and paediatric head injuries [1, 2, 4–6]. Previously a number of different summary measures of CPP, (e.g. means over hourly recordings or other time-frames, maximum or minimum values, percentage duration of derangement etc.), have been used as predictors of outcome. These are pragmatic and lose much of the detail by virtue of averaging the peaks and troughs of the perfusion pressure recording.

A measure is required that incorporates more than

one dimension (duration and degree), to give a more accurate quantification of the total burden of secondary insult. It should be equally applicable to children and adult monitoring.

This paper describes a novel Cumulative Pressure-Time index (CPT) that has been applied to a paediatric head injury dataset, and has been used to help determine critical thresholds for CPP in children.

Materials and methods

Sixty-six head-injured children (aged 2–16 years) from two regional UK centres had minute-to-minute recordings of physiological parameters including intracranial pressure (ICP), systolic, diastolic and mean arterial blood pressure (MAP), with automatically calculated CPP. Data were downloaded from bedside monitors and analysed off-line. Entry to this study included i) a post-resuscitation, pre-intubation Glasgow Coma Score (GCS) of 12 or less, or an Injury Severity Score of ≥ 16 in association with a GCS of 13–15 after head injury; ii) monitoring within 24 hours from the time of injury until removal of the ICP monitor.

Demographic data including age, gender, cause of injury, data and time of injury were collected, and a modified Glasgow Outcome Scale score was assigned at 6 months post-injury from a postal questionnaire completed by parents/carers or General Practitioners. Outcome was dichotomised into independent (GOS 4 & 5) and poor (GOS 1, 2 & 3) outcome, and alive vs. dead.

Pre-set levels of CPP derangement (i.e. values that were outside the normal range) were used for each year of age [4]. The 66 children were then grouped into 3 practical age-bands (2–6, 7–10, 11–16) such that the mean CPP for each band was within a 5 mmHg span, giving mean CPP threshold values of ≤ 48 mmHg for the 2–6 year olds, ≤ 54 mmHg for the 7–10 year olds, and ≤ 58 mmHg for the oldest group (11–16 years of age).

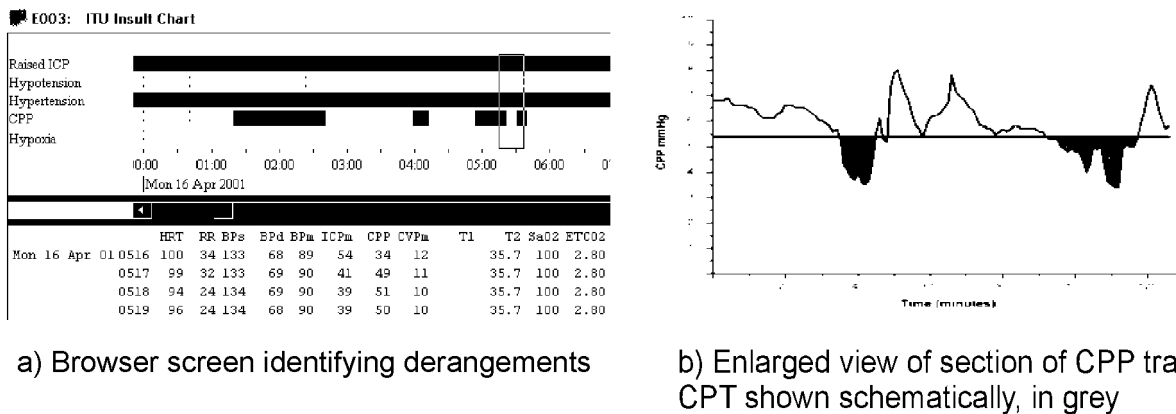


Fig. 1. Schematic representation of CPP and CPT: (a) The *Edinburgh Browser*[®] screen shows the recorded physiological values minute-by-minute below, and deranged physiology (i.e. >5 minutes) as horizontal black bars above. (b) A sample of the CPP trace from Fig. 1a is enlarged to illustrate the Cumulative Pressure-Time Index, shown as the area below the pre-selected threshold for CPP for age (horizontal line)

Method for calculating the CPT

Figure 1 outlines the steps in calculating the Cumulative Pressure-Time Index. Using the *Edinburgh Browser*[®] program [3] the minute-by-minute time series data were first validated to remove artefact and identify derangements lasting >5 minutes (Fig. 1a). Figure 1b shows a continuous trace of the resulting CPP data, with the selected threshold level. Each episode was identified and the summated areas between the CPP tracing and the threshold calculated.

This produced a single value representing both severity and duration of derangements. This can be shown mathematically by the following formula, where the greater the CPT value, the more CPP derangement was evident.

$$CPT = \sum (CPP_{\text{threshold}} - CPP) \times t_{\text{sample interval}}$$

The relationship between this new summary measure, and some alternative summary measures for CPP calculated from this dataset [i.e. the overall mean value for CPP (in mmHg); the accumulated total duration of all episodes of CPP derangement as specified by Browser as absolute time and as a percentage of the total CPP monitoring time] and outcome was explored.

Using the above, Receiver Operator Characteristic (ROC) curves were constructed. To test if there were significant differences between the ROC curves, 6 pairwise comparisons were made for each dichotomous outcome to establish which of the above summary measures were the best predictors of outcome.

The pre-set threshold levels for CPP for each age-band were then reduced by 10% and 20%, and the CPT recalculated for each, and related to outcome to identify which of the chosen thresholds were best discriminators of outcome.

Results

Of the 66 children, 47 suffered a severe head injury, 17 a moderate and 2 a mild head injury according to the entry criteria. There were 41 boys and 25 girls. Fifteen children were aged 2–6 years, 24 aged 7–10 years and 27 were in the oldest group (11–16 years). At 6 months outcome, 11 had died, none were vegetative,

6 were severely disabled (17 poor outcome), 19 were moderately disabled and 30 had made a good recovery (49 independent outcome).

CPT vs. outcome

The mean CPT values for those with poor and independent outcome were 31785 (mmHg × min) and 3391 respectively, and for the dead and alive were 48126 and 3220 respectively. Both were significantly predictive of outcome ($p < 0.001$). When the ROC curves were examined the areas under the curve for poor vs. independence and dead vs. alive were 0.839 (95% C.I. 0.731, 0.9470) and 0.957 (95% C.I. 0.901, 1.013) respectively.

CPT values by age vs. outcome

The CPT values for the 3 childhood age bands were also separately highly predictive for mortality ($p < 0.001$), and morbidity ($p = 0.027$, $p = 0.014$, and $p = 0.001$ for the 2–6, 7–10 and 11–16 year old groups respectively). The CPT derangement value was not significantly different (ANOVA) across the 3 childhood age bands for mortality and morbidity.

All summary measures related to outcome

A comparison of the other summary measures described above with the CPT index has been assessed utilising multiple ROC constructions (Fig. 2). The areas under the ROC curves for poor vs. independent outcome for the 4 measures were: – overall mean CPP

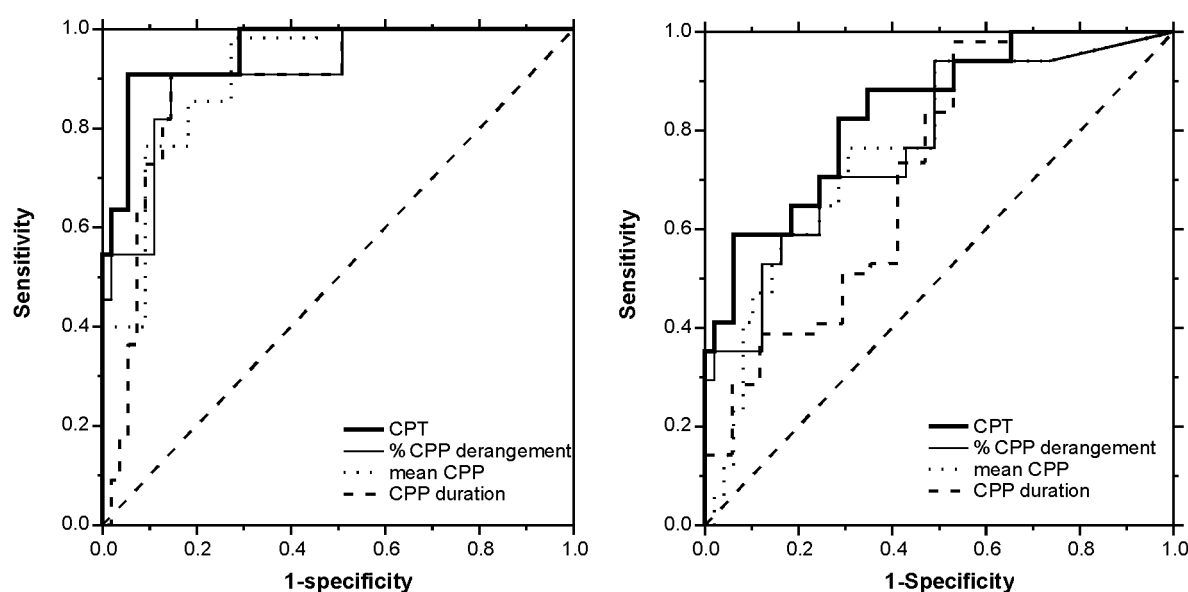


Fig. 2. ROCs for two dichotomised outcomes (death vs. survival on the left hand graph, and poor vs. independent outcome on the right hand graph) for CPP derangement when measured by i) overall Mean CPP in mmHg, ii) duration of CPP derangement; iii) derangement as a percentage of the acute monitoring time (% CPP derangement), and iv) using the Cumulative Pressure Time Index (CPT) for CPP

0.717; CPP insult duration 0.764; percentage CPP insult 0.776, and CPT at threshold 0.839.

Pairwise comparison of ROC curves for poor vs. independent outcome

The 6 possible pairwise comparisons were made of these ROC curves (MedCalc). This showed the CPT index was a significantly better measure than overall mean CPP ($p = 0.008$), CPP insult duration ($p = 0.02$), and percent duration of CPP ($p = 0.055$). None of the other possible paired comparisons were significant.

Pairwise comparison of ROC curves for dead vs. alive

Although all summary measures were highly predictive of mortality at 6 months, the area under the ROC curve for CPT index was 0.957, slightly greater than for the percentage insult duration (0.909), mean CPP (0.907) and CPP insult duration (0.886), but not statistically significant.

Threshold alteration and outcome

Although our chosen threshold levels above provide impressive ROC values, we considered whether a different CPP threshold might *even better* relate to outcome. Accordingly we carried out similar analyses of

Table 1. Values of the 'area under the ROC curves' for the cumulative pressure-time (CPT) at 'threshold CPP for age-band', 'threshold minus 10%', and 'threshold minus 20%' levels of CPP for mortality and morbidity

	Area under the ROC curve poor vs. independent outcome	Area under the ROC curve death vs. survival
CPP at 'threshold level'	0.839	0.957
Threshold minus 10%	0.818	0.974
Threshold minus 20%	0.809	0.975

CPT and ROC curves when the CPP threshold value was reduced by 10% and 20% (Table 1). For independent and poor outcome the area under the ROC curve for our threshold value was 0.839, greater than the value 0.818 for threshold minus 10%, and the value 0.809 for threshold minus 20%, but not significantly different.

For non-survival the area under the ROC curve did not improve prediction by reducing the threshold values.

Discussion

The CPT index that we have described takes into account both the severity and the duration of the physiological derangement of CPP and this index is highly

significantly related to outcome (classified as either independent vs. poor or alive vs. dead). This relationship to outcome also applies to children of different ages (2–6, 7–10 and 11–16 years of age). Our results show that there is a similar insult burden, measured by CPT, in young as in older children with head injury.

Previous studies on the relationship between CPP and outcome in head injury have normally used a uni-dimensional measure of the CPP insult, such as calculating the mean CPP value from the whole record, or the duration of reduced CPP, or the percentage time of the CPP reduction to the whole record, all of which we have similarly used. We have created a new index and demonstrated that the use of the CPT index at our threshold levels for children was a significantly better predictor of outcome.

Although CPT and the other indices were all significantly predictive of mortality, because of the absolute prediction of mortality with progressively lowered CPP values, there was no additional benefit from any one measure.

The threshold values chosen were inferred from physiological normative data [4] and it was not known at the outset whether those chosen values would prove to be definitive thresholds for sustaining brain “insult” (and be crucial to the determination of outcome). We therefore investigated alternative threshold levels of CPP, (of minus 10% and minus 20% below the physiological threshold), however these were not more predictive of outcome, indicating that the physiological threshold (after appropriate homeostatic compensation) best approximates the secondary brain insult threshold.

The brain insult may be acquired by either an exaggerated ICP or low MAP, and the CPT as a total CPP insult measure, will not recognise which is the major contributing cause. This is a common difficulty with any CPP measure.

Clearly the CPT index is a retrospective tool for use

in future clinical trials and retrospective studies, although it could theoretically be applied after 24 hours or other epochs of patient monitoring. It is likely that the age-specific critical threshold CPP value will be the practical value which will guide the clinician in the bedside care of the head injured child.

The CPP threshold values described are particularly useful for discriminating brain insult, but “treatment” or “intervention” thresholds, which would be arbitrary, would probably require higher target levels of CPP for treatment intervention in any formal clinical trial.

Acknowledgments

This research was supported by the Chief Scientist Office, (Scottish Office), U.K., Grant K/MRS/50/C2784, and the Northern Brain-wave Appeal.

References

1. Chambers IR, Treadwell L, Mendelow AD (2000) The cause and incidence of secondary insults in severely head-injured adults and children. *Brit J Neurosurg* 14: 424–431
2. Chambers IR, Treadwell L, Mendelow AD (2001) Determination of threshold levels of cerebral perfusion pressure and intracranial pressure in severe head injury by using receiver-operating characteristic curves: an observational study in 291 patients. *J Neurosurg* 94: 412–416
3. Howells TP (1994) *Edinburgh Monitor & Browser*® Computer Program
4. Jones PA, Andrews PJ, Easton VJ, Minns RA (2003) Traumatic brain injury in childhood: intensive care time series data and outcome. *Brit J Neurosurg* 17: 29–39
5. Jones PA, Andrews PJ, Midgley S, Anderson SI, Piper IR, Tocher JL, Housley AM, Corrie JA, Slattery J, Dearden NM (1994) Measuring the burden of secondary insults in head-injured patients during intensive care. *J Neurosurg Anesthesiol* 6: 4–14
6. Rosner MJ, Rosner SD, Johnson AH (1995) Cerebral perfusion pressure: Management protocol and clinical results. *J Neurosurg* 83: 949–962

Correspondence: Robert A. Minns, Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh, EH9 1UW, Scotland, UK. e-mail: Robert.Minns@ed.ac.uk

PAPER 4 (PUBLISHED)

**Chambers IR, Jones PA, Lo TYM, Forsyth R, Fulton B, Andrews PJD,
Mendelow AD, Minns RA.**

**Critical thresholds of intracranial pressure and cerebral perfusion
pressure related to age in paediatric head injury.**

***Journal of Neurology, Neurosurgery, and Psychiatry.* 2006; 77: 234-240.
doi: 1136/jnnp.2005.072215**

<http://jnnp.bmj.com/cgi/content/abstract/77/2/234?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&andorexactfulltext=and&searchid=1&FIRSTINDEX=0&sortspec=relevance&volume=77&firstpage=234&resource-type=HWCIT>

**Reproduced in accordance to the Authors' Licence for Publication of the
BMJ Group**



Critical thresholds of intracranial pressure and cerebral perfusion pressure related to age in paediatric head injury

I R Chambers, P A Jones, T Y M Lo, R J Forsyth, B Fulton, P J D Andrews, A D Mendelow and R A Minns

J. Neurol. Neurosurg. Psychiatry 2006;77;234-240; originally published online 15 Aug 2005;
doi:10.1136/jnnp.2005.072215

Updated information and services can be found at:
<http://jnnp.bmjjournals.com/cgi/content/full/77/2/234>

These include:

References

This article cites 20 articles, 3 of which can be accessed free at:
<http://jnnp.bmjjournals.com/cgi/content/full/77/2/234#BIBL>

Rapid responses

You can respond to this article at:
<http://jnnp.bmjjournals.com/cgi/eletter-submit/77/2/234>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Topic collections

Articles on similar topics can be found in the following collections

[Neurosurgery](#) (284 articles)
[Head injury](#) (306 articles)
[Other Pediatrics](#) (1689 articles)

Notes

To order reprints of this article go to:
<http://www.bmjjournals.com/cgi/reprintform>

To subscribe to *Journal of Neurology, Neurosurgery, and Psychiatry* go to:
<http://www.bmjjournals.com/subscriptions/>

PAPER

Critical thresholds of intracranial pressure and cerebral perfusion pressure related to age in paediatric head injury

I R Chambers, P A Jones, T Y M Lo, R J Forsyth, B Fulton, P J D Andrews, A D Mendelow, R A Minns



J Neurol Neurosurg Psychiatry 2006;**77**:234–240. doi: 10.1136/jnnp.2005.072215

See end of article for authors' affiliations

Correspondence to:
Dr Iain R Chambers,
Regional Medical Physics
Department, Newcastle
General Hospital,
Newcastle upon Tyne NE4
6BE, UK; i.r.chambers@
ncl.ac.uk

Received 16 May 2005
In revised form
10 August 2005
Accepted 10 August 2005
Published Online First
19 August 2005

Background: The principal strategy for managing head injury is to reduce the frequency and severity of secondary brain insults from intracranial pressure (ICP) and cerebral perfusion pressure (CPP), and hence improve outcome. Precise critical threshold levels have not been determined in head injured children.

Objective: To create a novel pressure–time index (PTI) measuring both duration and amplitude of insult, and then employ it to determine critical insult thresholds of ICP and CPP in children.

Methods: Prospective, observational, physiologically based study from Edinburgh and Newcastle, using patient monitored blood pressure, ICP, and CPP time series data. The PTI for ICP and CPP for 81 children, using theoretical values derived from physiological norms, was varied systematically to derive critical insult thresholds which delineate Glasgow outcome scale categories.

Results: The PTI for CPP had a very high predictive value for outcome (receiver operating characteristic analyses: area under curve = 0.957 and 0.890 for mortality and favourable outcome, respectively) and was more predictive than for ICP. Initial physiological values most accurately predicted favourable outcome. The CPP critical threshold values determined for children aged 2–6, 7–10, and 11–15 years were 48, 54, and 58 mm Hg, respectively.

Conclusions: The PTI is the first substantive paediatric index of total ICP and CPP following head injury. The insult thresholds generated are identical to age related physiological values. Management guidelines for paediatric head injuries should take account of these CPP thresholds to titrate appropriate pressor therapy.

Despite advances in resuscitation and trauma care the mortality and morbidity associated with head injury remains high.¹ If improvements are to be made in outcome from childhood head injury then a key challenge to neurointensive care is to minimise secondary ischaemic brain insults. Thresholds for intracranial pressure (ICP) and cerebral perfusion pressure (CPP) have become generally accepted in adult practice, although they have not been formally validated.^{2–9} There remains a pressing need to define critical thresholds in children that can be used to define levels for treatment intervention and insult detection in the intensive care environment. That these clinically important thresholds have not yet been established in children reflects the ever changing cardiovascular physiology during growth and development, which will alter thresholds with age until they approach adult values. There has also been a reluctance to define normal CPP values for different childhood age groups because of the lack of normative ICP data in growing children resulting from the changing intracranial dynamics.

The rationale for attempting to define critical thresholds of CPP was based on the findings of Jones *et al*¹⁰ who, using age specific norms, showed that the duration of age specific abnormal CPP predicted outcome (unfavourable/favourable) ($p = 0.004$) and mortality ($p = 0.003$) in a series of 71 children aged under 15 years. Similarly, Chambers *et al*¹¹ found important age related differences in CPP over the first six hours of monitoring, which were related to outcome.

Chambers *et al*⁴ have suggested a minimum target CPP value of 45 mm Hg in children, based on a single point from a receiver operating characteristic (ROC) curve that had been created using a minimum of a rolling mean CPP value in 84 children aged three months to 16 years. In a pilot study addressing the same issue, Jackson *et al*¹² calculated the CPP

insults determined for different arbitrary CPP thresholds (70, 60, and 50 mm Hg). For nine children aged less than 16 years, these median CPP thresholds were associated with median ICP values of 12, 14, and 24 mm Hg, and median blood pressure values were 77, 71, and 71 mm Hg, respectively.

All preceding studies that relate ICP/CPP to outcome in traumatic and non-traumatic encephalopathies in children have measured individual excursions of pressure, or the duration of derangement. Such approaches, which use a single summary measurement and do not combine severity and duration of derangement, may not capture the total insult burden. A measure that incorporates both degree and duration would theoretically be a better reflection of the total potential insult.

The sequential objectives of this study were, first, to create a novel index quantifying the secondary ischaemic brain insult, which combines both duration and intensity of derangement (for ICP and CPP), using detailed (one minute time resolution) physiological data; second, to derive age related physiological thresholds for ICP and CPP; and third, after establishing the sensitivity and specificity of the index in relation to outcome, to use it to define age related critical thresholds.

METHODS

This was a prospective observational study of 99 head injured children aged less than 16 years, admitted to two regional

Abbreviations: CPP, cerebral perfusion pressure; GOS, Glasgow outcome scale; ICP, intracranial pressure; ISS, injury severity score; PTI, pressure–time index; PTIC, pressure–time index for CPP; PTII, pressure–time index for ICP; ROC, receiver operating characteristic

Table 1 Criteria for enrolment

- Traumatic brain injury
- Post-resuscitation Glasgow coma score^{13 14} ≤ 12 or ≤ 15 , with injury severity score ≥ 16 or more
- Minute to minute computer data recording equipment available
- Physiological monitoring began within 24 hours of injury
- No previous head injury

centres in Edinburgh (n = 69) and Newcastle upon Tyne (n = 30) in 62 non-consecutive months up to July 2003. The study had local ethics committee and management approval in both centres and informed consent was obtained before enrolment in the study. The criteria for enrolment for entry to the study are given in table 1.

In all, 81 children (22 girls, 59 boys) aged two years or over (median 10.3 years, range 2 to <16) who fulfilled the criteria for entry into the study had ICP and arterial blood pressure monitoring. ICP and CPP treatment goals and general management guidelines were previously reported.¹⁰ These were as follows: age 0–13 years: CPP >50 mm Hg; ICP <15 mm Hg; age 14–15 years: CPP >60 mm Hg, ICP <20 mm Hg. The causes of injury are listed in table 2. There were 37 cases that had a surgical evacuation and 44 cases managed conservatively. The numbers of diffuse and focal injuries were 65 and 35, respectively, based on a Marshall computed tomography classification.

Outcome for all 81 children was recorded at six months post-injury using a questionnaire completed by parents, carers, or general practitioner. This was based upon the model of Adelson *et al*¹⁵ and allowed a modified Glasgow outcome scale (GOS) score to be assigned. In our analysis we used three different outcome dichotomies (table 3).

Monitoring of physiological variables

Intracranial pressure was monitored using an intraparenchymal transducer tipped catheter (Camino Laboratories, San Diego, California, USA) and continued for as long as was clinically indicated. Arterial blood pressure (systolic, diastolic, and mean) was monitored continuously using an intra-arterial line referenced to the right atrium. The bedside physiological monitors derived cerebral perfusion pressure. Oxygen saturation, heart rate, and body temperature (core and peripheral) were also recorded continuously.

Data acquisition and validation

Continuous recordings of variables at one minute time resolution were made from a networked paediatric intensive care unit in Newcastle and a mobile data collection system in Edinburgh. Both types of data recording were then transferred into the Edinburgh Browser[®] computer software system¹⁶ for later off-line validation, review, and analysis.

Invalid data were identified and discarded for various artefactual reasons such as detached probes, line flushing etc. Abnormal but valid data (e.g. during chest physiotherapy) were retained. Agonal data were retrospectively excluded for the final four hours and terminal readings were noted from the last available simultaneous measurement of ICP and CPP.

Because of the limited number of patients at each year age and the obvious physiological differences between the ages of 2 and 15 it was necessary to group children into clearly defined age bands. The age bands chosen were based upon physiological tables of blood pressure¹⁷ and by determining at what age the greatest incremental changes in blood pressure normally occurred. The age bands chosen were 2–6, 7–10, 11–15 years, and the lower limit means of mean arterial pressure (MAP) calculated for these age bands. These were considered to be the lowest acceptable value of MAP¹⁰ and are shown in Table 4.

Table 2 Causes of head injury in the 81 paediatric patients

Cause	Number
Pedestrian	38
Motor vehicle	11
Bicycle	10
Low fall	5
High fall	7
Struck on head	4
Assaults	3
Sport related	2
Penetrating injury	1

Table 3 Outcome dichotomies

1. **Favourable** outcome (good recovery or moderate disability) v **unfavourable** outcome (severe disability or death)
2. **Mortality** (death) v **survival** (good recovery, moderately disabled, or severely disabled)
3. **Morbidity** (good recovery) v **others** (moderately disabled, severely disabled, or dead)

Table 4 Predefined threshold values of intracranial pressure (ICP) and cerebral perfusion pressure (CPP)

Age	Mean MAP or CPP threshold
2–6 years	≤ 48 mm Hg
7–10 years	≤ 54 mm Hg
11–15 years	≤ 58 mm Hg

Given the relatively small contribution of ICP to the normal CPP value in young children, we chose the lowest acceptable CPP value to be the same as the lowest normal mean MAP value. This is likely to be numerically as accurate as formally estimating the true mean ICP level, even if data existed to allow such a calculation, and is described in detail in a previous study by Jones *et al*.¹⁰

Employing these values for each age band, the Edinburgh Browser system was then used to identify when the validated CPP value fell below or the ICP above these age related thresholds. Derangements were defined as abnormal values persisting for ≥ 5 minutes.¹⁸ The commencement, number, and duration of all derangements were identified.

Derivation of index

For each patient recording the duration of each CPP or ICP insult was detected by the Edinburgh Browser program, the difference between the specific age threshold and the recorded pressure value was calculated for each minute value. These were then summed to produce what we have termed the pressure–time index for CPP (PTIc) and for ICP (PTIi), which can be mathematically described by

$$\text{PTIc} = \sum (\text{CPP}_{\text{threshold}} - \text{CPP}_{\text{value}}) * t_{\text{sample interval}}/60$$

$$\text{PTIi} = \sum (\text{ICP}_{\text{value}} - \text{ICP}_{\text{threshold}}) * t_{\text{sample interval}}/60$$

A PTIc value of 40 mm Hg hours could represent a single insult of two hours' duration at a constant level of 20 mm Hg

below the threshold level. It could equally represent an insult lasting four hours where the CPP was 10 mm Hg below the threshold. In practice, insults are likely to vary considerably in both duration and depth, and the index will be a cumulative total of all such insults. Clearly the PTI will provide a valid comparison of total secondary insult between different patients only when either the data acquisition is limited to the point where the ICP values have been consistently stable or within the normal range, or when data acquisition is continued up to the point of death. Varying a cut off point between 0 and the maximum PTI value, we calculated the sensitivity and specificity of the index for PTI and PTIc for each of the three outcome divisions.

The sensitivity of PTIc will fall as the cut off point rises (fewer favourable outcomes with greater insult severity), while the specificity will rise with an increasing level of PTIc (greater number of unfavourable outcomes with increasing insult severity). Using the values of specificity and sensitivity calculated at each cut off point, ROC curves were plotted for each of the three outcome measures.

In order to investigate the effect of different threshold levels for insult detection and whether these might better differentiate outcome, each age band threshold was reduced first by 10% and then by 20%, and the PTIc recalculated. The age thresholds were then increased by 10% and the PTIc calculated again. Using the favourable versus unfavourable outcome dichotomy, ROC curves were plotted for each of the new threshold test levels and the area under each curve determined. For ICP the effect of altering the thresholds was also investigated by reducing the thresholds by 10% and then increasing them by 10% and then 20% (table 5).

The ICP monitoring was discontinued by the attending clinician when considered normal and no longer clinically appropriate. In order to ensure that the duration of monitoring did not significantly affect the PTI we have considered whether different durations or the severity of the primary injury would have influenced the PTI.

RESULTS

Based upon a post-resuscitation Glasgow coma score (GCS), 63 of the 81 children were considered to have suffered a severe head injury (GCS 3–8, E1, V = <2, and M = <5) and 16 children suffered a moderate head injury (GCS 9–12). There were two with a mild head injury (GCS 13–15, with injury severity score (ISS) ≥ 16). The mean ISS was 20 (range 9–38).

Of the 81 children, 35 made a good recovery, 30 were moderately disabled (65 favourable outcome), five were severely disabled at six months post-injury, and 11 died (16 unfavourable outcome). None remained in a vegetative state.

Cerebral perfusion pressure

Using the initial age specific physiologically based threshold values, the PTIc ranged from 0 to 1959 mm Hg hours. The median PTIc values varied significantly with GOS (good recovery 4.2; moderate disability 16.5; severe disability 73.6; dead 769.1 mm Hg hours, Kruskal–Wallis $p < 0.001$). There

was no significant difference in the mean values of PTIc between the three age groups taken as a whole (not subdivided by outcome)—that is, comparable amounts of insult were found in all childhood age groups.

Separately, within both favourable and unfavourable outcome, there were no significant differences in the magnitude of PTIc across the three age bands (fig 1A, $p = 0.3$). However, there was a very significant difference between the PTIc values in the unfavourable v favourable outcome categories ($p < 0.001$).

The PTIc value associated with an 80% sensitivity for a favourable outcome was 73.1 mm Hg hours. The corresponding values for mortality and morbidity were 331 and 1.4 mm Hg hours, respectively (fig 2A).

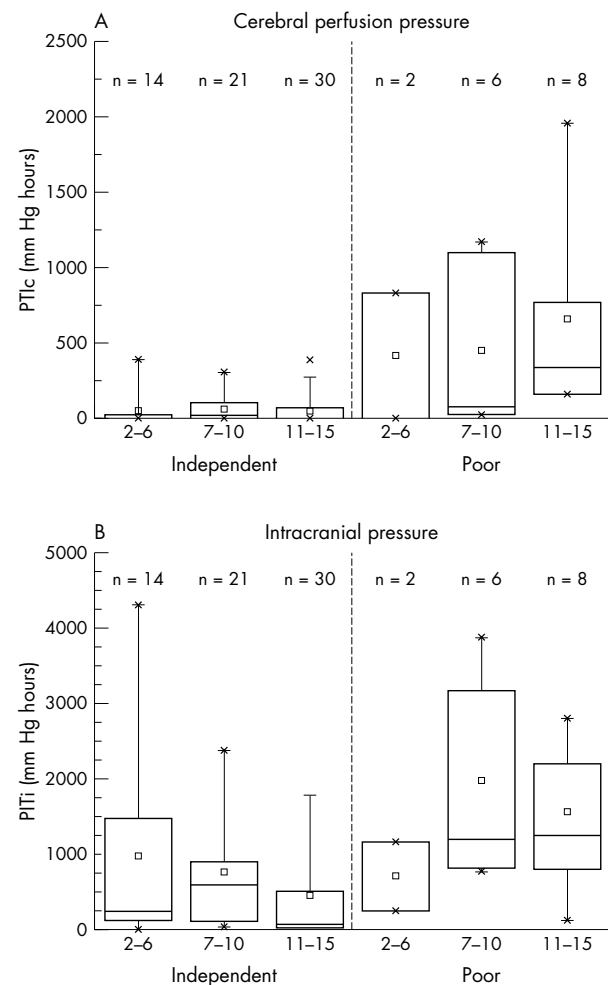


Figure 1 Box and Whisker plots of pressure–time index for (A) cerebral perfusion pressure (PTIc) and (B) intracranial pressure (PTIi) for the three age bands against outcome (favourable and unfavourable).

Table 5 Calculated ICP and CPP insult threshold values

Age (years)	CPP				ICP			
	–20%	–10%	Threshold	+10%	–10%	Threshold	+10%	+20%
2–6	38	43	48	53	5.8	6.4	7.0	7.7
7–10	43	49	54	59	7.9	8.7	9.6	10.5
11–15	46	52	58	64	11.7	13	14.3	15.6

CPP, cerebral perfusion pressure; ICP, intracerebral pressure.

When the threshold levels were changed (increased by 10% and lowered by 10% and 20%), the sensitivity fell more rapidly with lower threshold values, but there remained a clear delineation between each of the curves of sensitivity (fig 3A and B). For a specificity of 80% the PTIc values for each of the outcome groups were poorly separated, at 99.5, 96.9, and 101.4 mm Hg hours for mortality, independence, and morbidity, respectively, and with reduction in threshold values, the patterns were almost identical.

The ROC curves for PTIc created using the initial CPP threshold values for the three different outcome dichotomies are shown in fig 4A and the areas under the curves (AUCs) for all the ROC curves, along with their standard errors, are shown in table 6. The PTIc index had a very high predictive value for mortality (AUC = 0.957), and for a favourable outcome it was only slightly lower (AUC = 0.890). The predictive power was the lowest for separating the good outcome group from all the remaining outcome categories (AUC = 0.681).

When the threshold levels were altered by the previously described amounts, the resultant ROC curves for each of the three different outcome comparisons had smaller AUC values

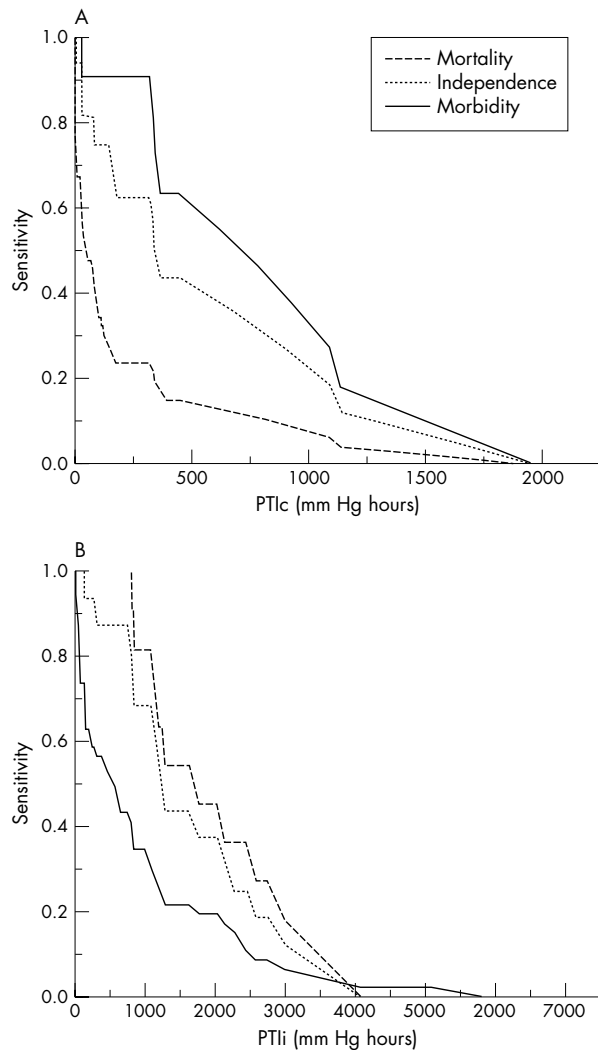


Figure 2 The sensitivities from the receiver operating characteristic (ROC) curve plotted against the pressure-time index for (A) cerebral perfusion pressure (PTIc) and (B) intracranial pressure (PTIi) in relation to mortality, favourable outcome, and morbidity.

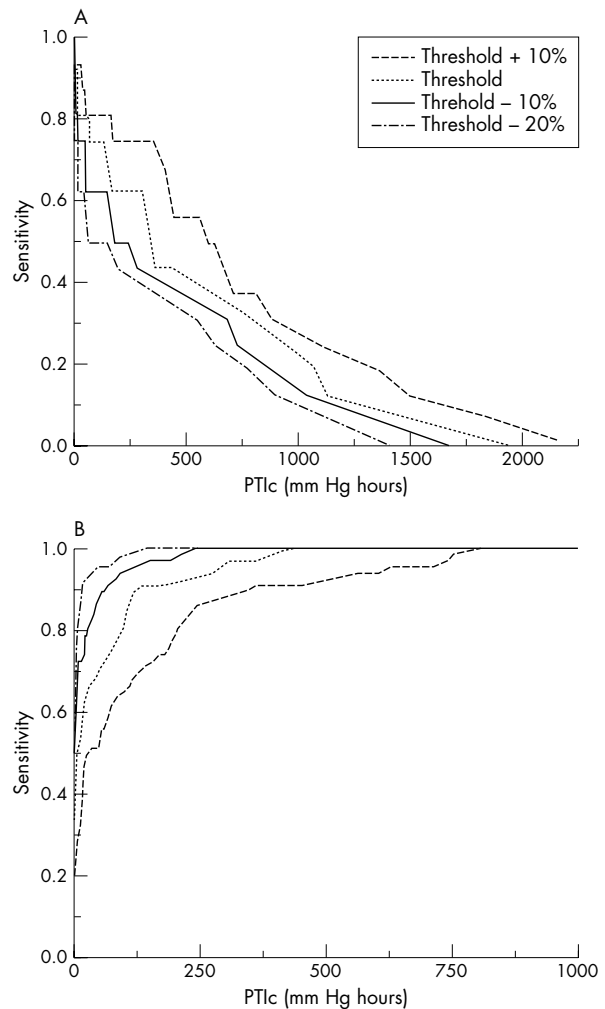


Figure 3 Sensitivity and specificity curves for the pressure-time index for cerebral perfusion pressure (PTIc) in relation to the tested threshold levels.

than that of our original threshold, indicating that our original thresholds were better predictive values.

For both favourable outcome and morbidity the original CPP threshold remained the best predictor of outcome, but for mortality the predictive value increased very slightly as the threshold level was decreased (table 6).

Intracranial pressure

The results for PTIi were similar but not identical to those for PTIc. The range of PTIi values was 0 to 5887 mm Hg hours, with a significant difference in median values of 232.8, 134.1, 776.3, and 1763.6 mm Hg hours in those who had good recovery, were moderately disabled, severely disabled, or died, respectively (Kruskal-Wallis, $p < 0.001$). There was a greater variation in the index across the two older age groups for both favourable and unfavourable outcome than for PTIc ($p = 0.026$ and $p < 0.001$) (fig 1B). There was a significant difference in the mean PTIi for favourable v unfavourable outcome ($p < 0.001$).

The rate of change in specificity was very similar to that of the CPP index for the three outcome comparisons. Although the shapes of the sensitivity curves were similar to those of PTIc, they were spread over a much wider range of values (fig 2B). For each of the three outcome dichotomies, the

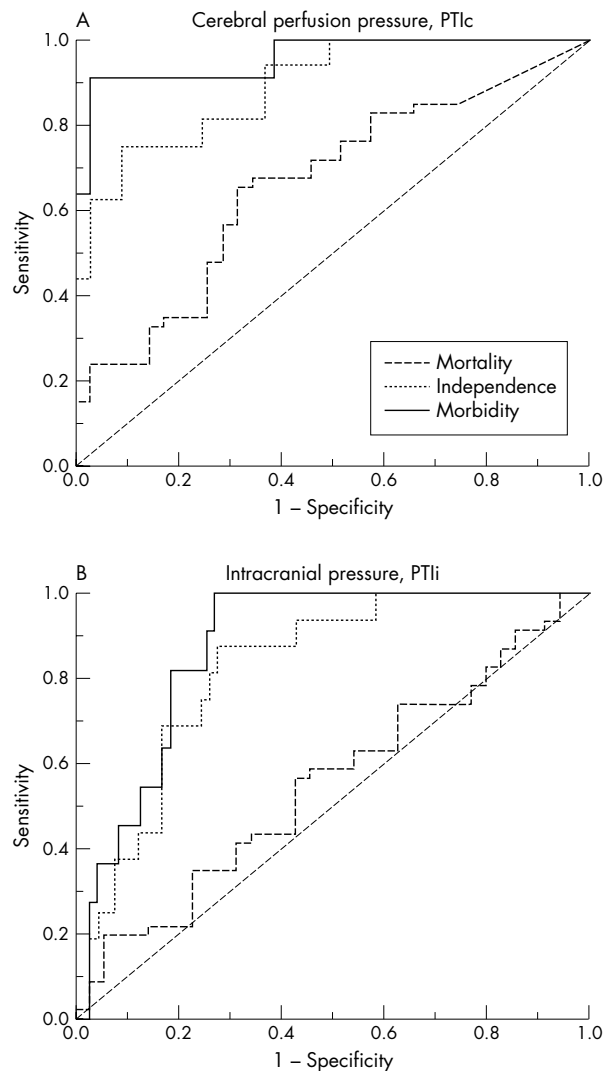


Figure 4 Receiver operating characteristic (ROC) curves for pressure-time index for (A) cerebral perfusion pressure (PTIc) and (B) intracranial pressure (PTIi) in relation to mortality, favourable outcome, and morbidity.

different threshold levels produced very similar sensitivity and specificity curves, with little difference between mortality, independence, and morbidity values—unlike those for CPP.

The areas under the ROC curves for the PTI for mortality, favourable outcome, and morbidity were smaller than the respective PTIc values (fig 4 and table 6). The PTIi index had a very high predictive value for mortality (AUC = 0.871) and a slightly lower value for favourable outcome (AUC = 0.819). For morbidity, the ROC curve stayed near to the diagonal, indicating that the predictive value was always close to 50%.

The terminal ICP values for children aged 2–6 years were 12 mm Hg, for 7–10, 18 mm Hg, and for 11–15, 16 mm Hg, corresponding to terminal CPP values of 78, 74, and 79 mm Hg, respectively. Although 42 children had terminal ICP values that were a mean of 11 mm Hg above their age specific thresholds, this was obviously compensated because there was a negligible amount of corresponding terminal CPP insult, with only five children having had terminal CPP values of a mean of 3 mm Hg below threshold levels. Even if this level of insult in these five patients continued for the

next hour, the PTI would still have estimated 98.6% of all CPP insult. It can be seen, therefore, that the pressure recordings had been discontinued only when there was virtually no ongoing CPP derangement.

The duration of ICP monitoring bore no relation to the severity of the primary injury, as assessed by the initial GCS motor score level ($p = 0.572$), nor to the ISS ($p = 0.237$). In addition, there was no significant difference between the two participating children's head injury units in the duration of ICP monitoring ($p = 0.749$).

DISCUSSION

For the clinician managing head injured patients, cerebral perfusion pressure is a crucially important variable for determining management decisions. We have developed a novel pressure-time index that, for cerebral perfusion pressure, is independent of age and has similar values across our three different age bands for a favourable outcome ($p < 0.02$) and survival ($p < 0.001$)—that is, insult occurs in all age groups and is always predictive of outcome, thus allowing comparability of CPP insults at any age. The PTI was therefore a measure solely of secondary brain insult and was independent of the duration of ICP monitoring and highly comparable between the two centres in this study.

This cohort of head injured children is similar with respect to the type, cause, and severity of injury and their outcome to other reported British and European case series of head injured children.^{19–23} Previous studies have focused on the relation between CPP and outcome in children's head injury, using a single measurement of derangement of CPP values (either mean, minimum, absolute, or percentage duration), but all failed to incorporate both duration and severity in the total burden of CPP insults.

Within the childhood population there are major differences between the blood pressure (and other physiological variables) of a 2 year old and a 10 year old, for example, unlike the adult population which recognises a single standard CPP value. However, the numbers of children in our study at each year from 2 to 15 are necessarily small and require banding for predictive statistics. The three age bands that we have used in determining insult thresholds have been described previously.¹¹ Although these bands could be refined, in practice the rate of development of the physiology of children can vary quite markedly and therefore the banding provides a measure of spread. We are conscious that we have not addressed ages between 0 and 2 years, because the rapidly changing physiology at this stage of development would require a larger cohort of patients and may only be possibly with a large multicentre study.

Cerebral perfusion pressure

The values of PTIc are significantly related to each of the GOS outcome categories or the combinations we have used (favourable *v* unfavourable, mortality, morbidity). Other studies cited above have shown a similar relation of “single dimension” CPP derangement to outcome. However, and uniquely, the PTIc is independent of age and was a very sensitive predictor of outcome, demonstrating a higher insult burden with worsened outcome on all three outcome measures, although the specificity proved to be less discriminating (that is, some children made a relatively good recovery with a high PTIc value).

The threshold levels that were chosen were based on physiological norms (2–6 years, 48 mm Hg; 7–10 years, 54 mm Hg; 11–16 years, 58 mm Hg) and were not known at the outset to be insult thresholds or treatment thresholds. We reduced the CPP thresholds arbitrarily by a factor of 10% and 20%, and similarly increased it by 10% (theoretical

Table 6 Areas under the receiver operating characteristic (ROC) curves for the pressure-time index of intracranial pressure and cerebral perfusion pressure at each of the threshold levels

Outcome dichotomy	Threshold level	ROC area	SE
Favourable v unfavourable	CPP+10%	0.858	0.054
	CPP	0.890	0.044
	CPP-10%	0.883	0.053
	CPP-20%	0.886	0.051
	ICP-10%	0.816	0.050
	ICP	0.819	0.050
	ICP+10%	0.823	0.050
	ICP+20%	0.825	0.052
Good recovery v the rest	CPP+10%	0.652	0.062
	CPP	0.662	0.061
	CPP-10%	0.645	0.061
	CPP-20%	0.667	0.060
	ICP-10%	0.555	0.065
	ICP	0.550	0.065
	ICP+10%	0.548	0.064
	ICP+20%	0.545	0.064
Mortality v the rest	CPP+10%	0.931	0.046
	CPP	0.957	0.034
	CPP-10%	0.968	0.027
	CPP-20%	0.966	0.026
	ICP-10%	0.864	0.042
	ICP	0.871	0.041
	ICP+10%	0.882	0.039
	ICP+20%	0.887	0.038

values), and our analysis showed that the performance of PTIc was robust.

ROC curves are a very suitable method for analysing a process that has a binary outcome. The area under the curve gives a measure of the predictive value of a test and can be used to compare different variables.²⁴ Our ROC analysis has clearly shown that the PTI value for CPP, using our original CPP threshold values (at different ages), most accurately separated the outcome categories. Theoretical threshold levels above or below were less predictive than the original physiologically based thresholds.

Intracranial pressure

It is generally accepted that raised intracranial pressure is a secondary insult that adversely effects outcome.^{4, 25, 26} For ICP our new index has an ROC area under the curve of 0.871 (for mortality), and 0.819 (favourable v unfavourable), and although this is lower than the CPP value it clearly has a considerable relation to outcome.

The children who were severely disabled or died had progressively larger amounts of ICP insult, as would be expected, but there was an apparently anomalous finding of median PTIi values in those children who made a good recovery (232.8 mm Hg hours) compared with those who were moderately disabled, who had less insult (134.1 mm Hg hours). However, this represents only a 6% difference in the total PTIi, and may be a reflection of several outliers. Investigating this, we found there was no significant difference in the duration of monitoring between those with a good recovery and those with moderate disability, but the significant differences in the mean values of PTIi between the three age groups ($p = 0.017$) might suggest that the a priori ICP threshold levels do not equate to the insult thresholds as they are too low.

Increasing or decreasing the ICP thresholds by 10% or 20% (of a relatively small numerical ICP value) did not significantly change the sensitivity or specificity and hence did not improve the predictive value in relation to outcome—that is,

it is the CPP at our age specific thresholds that is the more influential factor in the outcome of paediatric head injury.

Critical thresholds

Using this new index (PTI), which combines the degree and duration of derangement, to quantitate the totality of brain insult from pressure derangement, and when applied to predefined insult threshold values for ICP and CPP, we have shown that the index is robust and relates extremely well to both morbidity and favourable outcome. We have tested limits of these physiological thresholds by both increasing and decreasing them, and have shown clearly by calculating sensitivity and specificity of PTI that our initial physiological thresholds best separated children with head injuries for both mortality and favourable outcome using CPP. These critical threshold values for children aged 2–6, 7–10, and 11–<16 years were 48, 54, and 58 mm Hg, respectively. In relation to ICP, the predictive value of the PTI index improved slightly as the threshold level was increased. This may have been because the initial values, although developed from normal age related values, were at a relatively low level, but even a 20% change did not bring about a large absolute difference.

This is the first study that includes more than one dimension in the analysis of ICP and CPP data collected from head injured children. We consider that the PTI will therefore be needed for future studies that determine the totality of cerebral perfusion pressure insults. Further work is required to establish whether the shorter more severe insults have comparable effects to longer but less severe derangements.

There may, in theory, be three different types of threshold: physiological thresholds, treatment thresholds, and brain insult thresholds. Each of these would need to be precisely defined and understood, but we believe that for children the physiological thresholds (of CPP) are identical to the insult thresholds. Treatment thresholds are likely to be more arbitrary and individual, and what is defined here are absolute CPP values below which secondary brain injury

has a significant impact of the injured child's outcome. Critical care guidelines emphasise the value of ICP monitoring, and a more exact knowledge of the level of damaging thresholds of ICP and CPP should enhance the ability of the clinician to recognise abnormal pressures and to take therapeutic steps to avoid the insult extending beyond these thresholds.

ACKNOWLEDGEMENTS

We are grateful to the children, parents, and all staff of the intensive care units. We would like to acknowledge the help of Dr Barbara Gregson for statistical advice. This study was funded by the Chief Scientist Office, Scotland Grant K/MRS/50/C2784 and the Northern Brainwave Appeal for monitoring equipment.

Authors' affiliations

I R Chambers, Regional Medical Physics Department, Newcastle General Hospital, Newcastle upon Tyne, UK

R J Forsyth, Department of Paediatrics, Newcastle General Hospital

B Fulton, Department of Anaesthetics, Newcastle General Hospital

A D Mendelow, Department of Neurosurgery, Newcastle General Hospital

P A Jones, **T Y M Lo**, **R A Minns**, Child Life and Health, University of Edinburgh, Royal Hospital for Sick Children, Edinburgh, UK

P J D Andrews, Department of Anaesthetics, Western General Hospital, Edinburgh

Competing interests: none declared

REFERENCES

- 1 Sharples PM, Aynsely-Green A, Eyre JA. Avoidable factors contributing to the death of children with head injury. *BMJ* 1990;**300**:87-91.
- 2 Rosner MJ, Rosner SD, Johnson AH. Cerebral perfusion pressure: management protocol and clinical results. *J Neurosurg* 1995;**83**:949-62.
- 3 Chambers IR, Treadwell L, Mendelow AD. The cause and incidence of secondary insults in severely head-injured adults and children. *Br J Neurosurg* 2000;**14**:424-31.
- 4 Chambers IR, Treadwell L, Mendelow AD. Determination of threshold levels of cerebral perfusion pressure and intracranial pressure in severe head injury by using ROC curves: an observational study in 291 patients. *J Neurosurg* 2001;**94**:412-16.
- 5 Chesnut RM, Marshall SB, Piek J, et al. Early and late systemic hypotension as a frequent and fundamental source of cerebral ischaemia following severe brain injury in the Traumatic Coma Data Bank. *Acta Neurochir Suppl (Wien)* 1993;**59**:121-5.
- 6 Dearden NM. Mechanisms and prevention of secondary brain damage during intensive care. *Clin Neuropathol* 1998;**17**:221-8.
- 7 Maas AR, Dearden M, Teasdale GM, et al. EBIC-guidelines for management of severe head injury in adults. *Acta Neurochir* 1997;**139**:286-94.
- 8 BTF/AANS. Management and prognosis of severe traumatic brain injury. A joint project by the Brain Trauma Foundation and the American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care, 2000. ISBN 0-9703144-0-X.
- 9 SIGN Guidelines No 46. Early management of patients with head injury, Scottish Intercollegiate Guidelines Network (SIGN), 28 Thistle Street Edinburgh EH2 1EN (www.sign.ac.uk), 2000.
- 10 Jones PA, Andrews PJ, Easton VJ, et al. Traumatic brain injury in childhood: intensive care time series data and outcome. *Br J Neurosurg* 2003;**17**:29-39.
- 11 Chambers IR, Stobart L, Jones PA, et al. Age-related differences in intracranial pressure and cerebral perfusion pressure in the first 6 hours of monitoring after children's head injury: association with outcome. *Childs Nerv Syst* 2005;**21**:195-9.
- 12 Jackson S, Piper IR, Wagstaff A, et al. Quantification of cerebral perfusion pressure (CPP) "secondary insults" in children: a study of the effects of using different CPP thresholds. *Br J Neurosurg* 1999;**13**:104.
- 13 Teasdale GM, Jennett B. Assessment of coma and impaired consciousness. A practical scale. *Lancet*, 1974;ii, 81-94.
- 14 Reilly PL, Simpson DA, Sprad R, et al. Assessing the conscious level in infants and young children: a paediatric version of the Glasgow Coma Scale. *Childs Nerv Syst* 1988;**4**:30-3.
- 15 Adelson PD, Clyde B, Kochanek PM, et al. Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. *Pediatr Neurosurg* 1997;**26**:200-7.
- 16 Howells T. Edinburgh Monitor and Browser; computer programme 1994.
- 17 NHLBI. Report of the second task force in blood pressure control in children, 1987. Bethesda, Maryland: National Heart, Lung and Blood Institute, 1987.
- 18 Jones PA, Andrews PJ, Midgley S, et al. Measuring the burden of secondary insults in head-injured patients during intensive care. *J Neurosurg Anaesth* 1994;**6**:4-14.
- 19 Abbate B, Donati P, Cagnoni G. Head injuries in children. Considerations on 3715 consecutive cases. *Minerva Pediatr* 2000;**52**:623-38.
- 20 Emanuelson I, Wendt L. Epidemiology of traumatic brain injury in children and adolescents in south-west Sweden. *Acta Paediatr* 1997;**86**:730-5.
- 21 Jennett B. Epidemiology of head injury. *Arch Dis Child* 1998;**78**:403-6.
- 22 Minns RA, Lo TY. Head and neck injuries. In: Keeling JW, Busuttill A, editors. *Paediatric forensic medicine and pathology*. London: Edward Arnold, 2005;(in press).
- 23 Brookes M, MacMillan R, Cully S, et al. Head injuries in accident and emergency departments. How different are children from adults? *J Epidemiol Community Health* 1990;**44**:147-51.
- 24 Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operator characteristic (ROC) curve. *Radiology* 1982;**143**:29-36.
- 25 Marmarou A, Anderson RL, Ward JD, et al. Impact of ICP instability and hypotension on outcome in patients with severe head trauma. *J Neurosurg* 1991;**75**(suppl):S59-66.
- 26 Miller JD, Becker DP, Ward JD, et al. Significance of intracranial hypertension in severe head injury. *J Neurosurg* 1977;**47**:503-16.

PAPER 5 (PUBLISHED – ONLINE CASE REPORT)

Lo TYM, Jones PA, Freeman JA, McFadzean J, Minns RA.

The role of High Frequency Oscillatory Ventilation (HFOV) in the management of children with severe traumatic brain injury (TBI) and concomitant lung pathology where conventional ventilation had failed.

Pediatric Critical Care Medicine. 2008; 9(5):e38-e42.

<http://www.pccmjournal.org/pt/re/pccm/abstract.00130478-200809000-00023.htm;jsessionid=JpYFSZhv1gC015Jsyc2PPGwYVjcp8hSDGgy40YBNKQ3CxrJr9Jvy!1285625425!181195628!8091!-1>

PAPER 6 (PUBLISHED)

***Lo TYM, Jones PA, Chambers IR, Beattie TF, Forsyth R, Mendelow AD,
Minns RA.***

**Modulating effect of Apolipoprotein E polymorphisms on secondary
insult and outcome after childhood brain trauma.**

***Child's Nervous System.* 2009; 25: 47 – 54.**

doi: 10.1007/s00381-008-0723-4

Reproduced with the Publisher's Permissions.

Modulating effect of apolipoprotein E polymorphisms on secondary brain insult and outcome after childhood brain trauma

T. Y. M. Lo · P. A. Jones · I. R. Chambers ·
T. F. Beattie · R. Forsyth · A. D. Mendelow ·
R. A. Minns

Received: 22 July 2008 / Published online: 7 October 2008
© Springer-Verlag 2008

Abstract

Objective The aim of this study was to determine the relationship between apolipoprotein E (APO E) alleles, the amount of cerebral perfusion pressure (CPP) insult and outcome in children after brain trauma.

Materials and methods In a prospective two-centre case–control study, the APO E genotypes of 65 critically ill children admitted after brain trauma were correlated with age-related CPP insult quantification, conscious state at the time of discharge from intensive care and global outcome at 6 months post-injury. One hundred sixty healthy age- and sex-matched children were genotyped as controls.

Results The CPP insult level among the e4 carriers with poor outcome was significantly less than the non-e4 carriers ($p=0.03$). Homozygotic e3 patients with good recovery did so despite having suffered nearly 26 times more CPP insult than those who were not e3 homzygous ($p=0.02$).

Conclusion Different APO E alleles may potentially affect cerebral ischaemic tolerance differently in children after brain trauma.

Keywords Traumatic brain injury · Apolipoprotein E genetic polymorphisms · Cerebral perfusion pressure · Outcome · GCS · Paediatrics

Introduction

Cerebral perfusion pressure (CPP) insult is a proven determinant of outcome after brain trauma in children [1, 2] and adults [3, 4]. Despite vigilant brain trauma management to minimise CPP insult, outcome of childhood traumatic brain injury (TBI) remains diverse and sometimes appears to be out of proportion to the severity of the primary brain injury and the amount of secondary brain insult experienced. This suggests that there may be other influences, for example genetic makeup, which might be an additional determinant of outcome following brain trauma.

The polymorphic Apolipoprotein E (APO E) gene, located on chromosome 19, has been reported to affect outcome after brain trauma in adults [5–7]. Teasdale et al. [8] have recently suggested that its influence on the recovery of childhood head injury may be even more important. However, the mechanisms through which the APO E genetic polymorphisms affect outcome remain unknown. Postulated mechanisms have originated mostly from animal or in vitro investigations and include potential allelic-related differential influence on oxidative stress [9] or excitotoxicity [10, 11] and the rate of conversion of the neuroprotective β amyloid precursor protein (β APP) to the neurotoxic β amyloid (β A4) [12], but the clinical relevance of these mechanisms remains unclear. Despite the vast literature supporting the predictive value of CPP insult on brain trauma outcome in adults and children, the relationships between APO E genotypes and

T. Y. M. Lo (✉) · P. A. Jones · R. A. Minns
Section of Child Life & Health, University of Edinburgh,
20 Sylvan Place,
Edinburgh EH 9 1LF, UK
e-mail: mils.lo@doctors.org.uk

T. Y. M. Lo · T. F. Beattie · R. A. Minns
Royal Hospital for Sick Children,
Edinburgh, UK

I. R. Chambers · R. Forsyth · A. D. Mendelow
Newcastle General Hospital,
Newcastle-upon-Tyne, UK

CPP insult following brain trauma has not been assessed previously.

This study aimed to determine the APO E allelic distributions in critically ill children following brain trauma and normal control children and to assess whether possessions of the various APO E alleles differentially affect the amount of CPP insult and outcome in childhood brain trauma.

Materials and methods

Participants

Sixty-five children requiring neuro-intensive care post-brain trauma were enrolled into a prospective two-centre case-controlled study over a two and a half-year study period. Forty-five children had continuous intracranial pressure (ICP) monitoring, but seven were excluded from this subgroup analyses because significant amounts of ICP data were lost during the first 24 h post-injury from computer downtime. Control subjects comprised 160 attendees at the emergency department follow-up clinic following minor injuries (excluding head injuries) and were age- and sex-matched to the brain-injured children. The study was approved by the local ethics and hospital research management committees. Parental consent was obtained for inclusion in the study.

APO E genotyping

Buccal smears were collected from all participants for DNA extraction using the commercially available PUREGENE DNA Buccal Smear Isolation Kit™ (produced by Gentra Systems). The extracted DNA was used for subsequent APO E genotyping employing polymerase chain reaction methodology, restriction enzyme (HhaI) digestion and metaphor agarose gel electrophoresis adapted from Hixson and Vernier [13].

Quantification of CPP insult and outcome

In brain-injured children, routinely monitored physiological parameters in minute resolutions were prospectively downloaded from the PICU bedside monitors for detection of age-specific physiological derangements as described previously using the Edinburgh Browser programme [2]. The total burden of CPP insult was quantified using the cumulative pressure time index for CPP (PTIc) [14] in patients with ICP monitoring.

Conscious level was determined at PICU discharge which equated to the end of neuro-intensive care when the brain-injured children no longer required airway protection, ventilatory and circulatory support. The modified Glasgow Coma Scale (GCS) was used for this assessment, and the children were dichotomised to those

who had ‘regained consciousness’ (GCS > 8) and those with ‘delayed return of consciousness’ (GCS 8 or less). Global outcome was assessed at 6 months post-injury using the modified Glasgow Outcome Score (GOS) and dichotomised into ‘good recovery’ when GOS 4 and 5 was achieved and ‘poor outcome’ when GOS was between 1 and 3.

Analyses

Comparisons of the APO E allelic distribution ratios were made among brain-injured children, their controls and a previously reported cohort of healthy Scottish adults ($n=400$) [15]. Chi-square tests were employed to assess any statistical difference. To determine whether children with different allelic groups experienced different amounts of CPP insult, the patients were divided into the following dichotomies for analyses: children with the e2 allele vs those without; children carrying the e4 allele vs the non-e4 carriers and the e3 homozygous vs the non-e3 homozygous. Mann–Whitney U tests were used to detect any statistical significance. Relationships between the various outcome dichotomies and different APO E allelic groups detailed above were assessed using Fisher’s exact tests.

Results

Demographics

The subgroup of children with CPP insult measurement had more severe brain injury and longer ICU duration of stay than those of the whole cohort, but other demographic details such as age, sex distribution and initial brain computed tomography findings did not otherwise differ significantly. The demographic details were similar between the different allelic groups.

APO E allelic distribution

APO E genotyping was successful in all participants. The APO E allelic distributions for brain-injured children and their controls are summarised in Table 1. The distribution ratios of the three APO E alleles were similar between the brain-injured children and their controls, but when compared with healthy adults from a previously reported Scottish population [15], the e2 allele was significantly overrepresented ($p=0.04$) among our participants (Table 1).

APO E alleles and outcome

Of the whole brain trauma cohort, 46 children ‘regained consciousness’, whilst 19 remained in a coma at the time of PICU discharge. At 6 months post-injury, eight children had

Table 1 APO E Allelic distributions

	APO E alleles				APO E genotypes					
	e2	e3	e4	Total	e2/e2	e2/e3	e2/e4	e3/e3	e3/e4	e4/e4
TBI Children (whole cohort, $n=65$)	18 (0.14)	96 (0.74)	16 (0.12)	130	2	11	3	38	9	2
Control children ($n=160$)	36 (0.11)	238 (0.74)	46 (0.15)	320	3	26	4	90	32	4
χ^2 for trend: e2 $p=0.44$; e3 $p=0.91$; e4 $p=0.86$										
TBI children (whole cohort $n=65$)	18 (0.14)	96 (0.74)	16 (0.12)	130						
TBI children (CPP insult measured, $n=38$)	13 (0.15)	53 (0.73)	10 (0.12)	76	1	8	3	20	5	1
χ^2 for trend: e2 $p=0.53$; e3 $p=0.53$; e4 $p=0.86$										
TBI children (whole cohort $n=65$)	18 (0.14)	96 (0.74)	16 (0.12)	130						
Healthy adults [15] ($n=400$)	66 (0.08)	616 (0.77)	118 (0.15)	800	2	51	11	233	99	4
χ^2 for trend: e2 $p=0.04$; e3 $p=0.43$; e4 $p=0.46$										

Brain trauma children and their controls have similar distribution ratios for the three APO E alleles, but a significant overrepresentation of the e2 allele ($p=0.04$) is noted among our participants when they are compared with healthy adults from a previously reported Scottish population [15]

‘poor outcome’, of whom only one had ‘regained consciousness’ at PICU discharge ($p<0.001$, Fisher’s exact test).

Table 2 summarises the relationships between the different allelic dichotomies and outcome at PICU discharge and 6 months post-injury. Only three of the 38 e3 homozygous (8%) had ‘poor outcome’ at 6 months post-injury, whilst three of the 16 e2 carriers (19%) and three of the 14 e4 possessors (21%) had ‘poor outcome’. There was a trend for the e2 allele possessors to remain in a coma at PICU discharge ($p=0.05$, Fisher’s exact test; Table 2).

APO E alleles and CPP insult

Children with various APO E alleles experienced differential amounts of CPP insult, with e3 homozygous experiencing the most whilst e4 carriers had the least CPP insult. Children carrying the e4 alleles had significantly less (13.3 times) CPP insult than those without the e4 allele ($p=0.04$, Mann–Whitney U test; Fig. 1). E3 homozygous suffered 9.2 times more CPP insult than the non-e3 homozygous ($p=0.03$, Mann–Whitney U test; Fig. 1). In general, children in possession of the e2 allele experienced 2.2 times less CPP

insult than non-e2 carriers, but this did not reach statistical significance ($p=0.58$, Mann–Whitney U test; Fig. 1).

APO E alleles, CPP insult and outcome

At PICU discharge

Children with delayed recovery of consciousness at PICU discharge tended to have experienced more CPP insult than those who regained consciousness early with the exception of those who were e3 homozygous where little variation of CPP insult was found (Table 3).

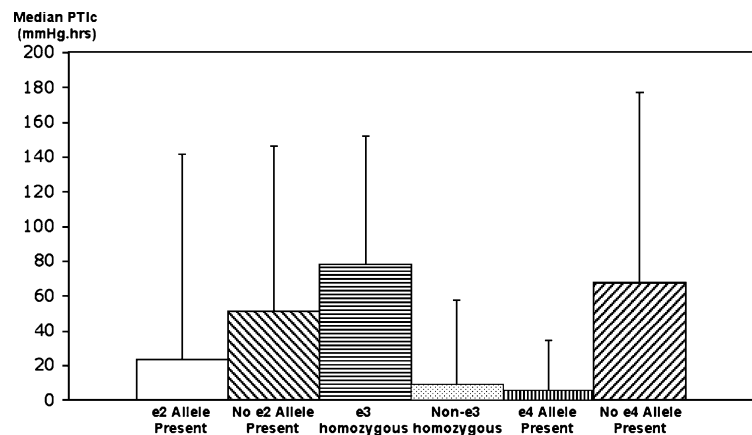
When considering children who regained consciousness (GCS >8) at PICU discharge, e3 homozygous had suffered significantly more CPP insult than non-e3 homozygous ($p<0.01$, Mann–Whitney U test), whilst children with the e4 allele experienced significantly less CPP insult than those without the e4 allele ($p=0.03$, Mann–Whitney U test; Table 4). There was a trend suggesting that e2 carriers who had ‘regained consciousness’ had suffered less CPP insult than those without the e2 allele ($p=0.05$, Mann–Whitney U test; Table 4).

Table 2 APO E Allelic group and outcome assessment dichotomies

	Regained consciousness ($n=46$)	Delayed return of consciousness ($n=19$)	Significance (Fisher’s exact test)	Good recovery ($n=57$)	Poor outcome ($n=8$)	Significance (Fisher’s exact test)
Whole cohort						
e2 allele present	8	8	$p=0.05$	13	3	$p=0.40$
No e2 allele present	38	11		44	5	
e3 homozygous	30	8	$p=0.10$	35	3	$p=0.26$
Non-e3 homozygous	16	11		22	5	
e4 allele present	8	6	$p=0.32$	11	3	$p=0.35$
No e4 allele present	38	13		46	5	

There is a trend for the e2 allele possessors to remain in a coma at PICU discharge ($p=0.05$, Fisher’s exact test)

Fig. 1 The e3 homozygous had more CPP insult than the non-e3 homozygous ($p=0.03$, Mann–Whitney U test), whilst the e4 carriers had less insult than the non-e4 carriers ($p=0.04$, Mann–Whitney U test)



6 months post-injury

At 6 months post-injury, regardless of the APO E genotypes, brain-injured children with a good recovery had suffered less CPP insult than those who had poor outcome (Table 3). When considering children with a good recovery, e3 homozygous patients recovered well despite having suffered significantly (nearly 26 times) more CPP insult during their ICU management than those who were not e3 homozygous ($p=0.02$, Mann–Whitney U test; Table 4). Children possessing the e4 allele with a poor outcome experienced significantly less CPP insult than those without the e4 allele ($p=0.03$, Mann–Whitney U test; Table 4). Furthermore, the median PTIC for the e4 carriers with poor outcome was 22.8 mmHg h, which

should have conferred good recovery given that the median PTIC for the whole cohort with good recovery was 32.3 mmHg h (Table 3).

Discussion

This study evaluated the association between APO E genotypes, the measured CPP insult after paediatric brain trauma, conscious state at PICU discharge and outcome at 6 months post-injury and demonstrated that children carrying various APO E alleles experienced different amounts of CPP insult following brain trauma. Additionally, we established an e2 allelic overrepresentation among our exclusive child cohort.

Table 3 APO E allelic groups, cerebral perfusion pressure insult, and outcome dichotomies

	Median PTIC (ranges, mmHg h)		Significance (Mann–Whitney U)	Good recovery	Poor outcome	Significance (Mann–Whitney U)
	Regained consciousness	Delayed return of consciousness				
Whole group	33.03 (0.00–273.22) ($n=22$)	60.83 (0.00–1169.27) ($n=16$)	$p=0.30$	32.33 (0.00–388.58) ($n=31$)	150.20 (5.10–1169.27) ($n=7$)	$p=0.04$
e2 allele present	2.22 (0–13.77) ($n=4$)	82.47 (0–1169.27) ($n=8$)	$p=0.06$	4.43 (0.00–121.28) ($n=9$)	150.20 (48.98–1169.27) ($n=3$)	$p=0.03$
e3 homozygous	75.26 (2.27–273.22) ($n=14$)	79.95 (0.00–830.40) ($n=6$)	$p=0.93$	70.13 (0.00–388.58) ($n=18$)	495.86 (161.32–830.40) ($n=2$)	$p=0.04$
e4 allele present	1.33 (0.00–11.93) ($n=4$)	22.83 (0.00–115.95) ($n=5$)	$p=0.14$	1.33 (0.00–115.95) ($n=6$)	22.83 (5.10–48.98) ($n=3$)	$p=0.20$

Children with poor outcome have significantly more CPP insult than those with good recovery ($p=0.04$). This is also true for children carrying the e2 allele ($p=0.03$) and the e3 homozygous children ($p=0.04$)

Table 4 APO E allelic dichotomies, cerebral perfusion pressure insult and outcome. Median PTIC values (ranges, mmHg h) are given

	Regained consciousness (PICU discharge)	Good recovery (6 months post-injury)	Poor outcome (6 months post-injury)
e2 Present	2.22 (0.00–13.97) (n=4)	4.43 (0.00–121.28) (n=9)	150.20 (48.98–1,169.27) (n=3)
No e2 present	51.17 (0.00–273.22) (n=18)	51.17 (0.00–388.58) (n=22)	92.08 (5.10–830.40) (n=4)
<i>p</i> value	0.05	0.23	0.48
e3 Homozygous	75.26 (2.23–273.22) (n=14)	70.13 (0.00–388.58) (n=18)	495.86 (161.32–830.40) (n=2)
Non-e3 homozygous	1.33 (0.00–13.77) (n=10)	2.67 (0.00–121.28) (n=13)	48.98 (5.10–1,169.27) (n=5)
<i>p</i> value	<0.01	0.02	0.25
e4 Present	1.33 (0.00–11.93) (n=4)	1.33 (0.00–115.95) (n=6)	22.83 (5.10–48.98) (n=3)
No e4 allele	51.17 (0.00–273.22) (n=18)	49.95 (0.00–488.58) (n=25)	495.86 (150.20–1,169.27) (n=4)
<i>p</i> value	0.03	0.09	0.03

For patients who have regained consciousness at PICU discharge, the e4 allelic carriers have experienced significantly less CPP insult than the non-e4 carriers ($p=0.03$), whilst e3 homozygotic children have experienced significantly more CPP insult than those who are not e3 homozygous ($p<0.01$). For patients with poor outcome at 6 months, significantly less CPP insult is found among those carrying the e4 allele when compared to those without the e4 allele ($p=0.03$). E3 homozygotic children with good recovery at 6 months post-injury have suffered significantly more CPP insult than those who are not e3 homozygous ($p=0.02$)

Differential CPP insult between the carriers of the different APO E alleles

Over the last decade, growing numbers of reports have described the association between the APO E e4 allele and poorer outcome after brain trauma in adults [5–7, 16], but its relationships with secondary CPP insult and influence on outcome after childhood brain trauma has not been previously investigated. Our study confirms the importance of CPP insult on outcome after childhood brain trauma regardless of the patient's APO E status. Additionally, this study was the first to report children carrying the e4 allele to have experienced the least amount of CPP insult, whilst the e3 homozygotic patients suffered the most after brain trauma despite having sustained primary brain injury of similar severity and being treated with standardised intensive care management. This suggests that APO E genetic polymorphisms may have a potential differential influence on the body's response to primary brain injury or its management.

Raised ICP, arterial hypotension or a combination of both may cause low CPP. Post traumatic cerebral swelling is an important cause of intracranial hypertension, and its severity in experimental brain trauma may be modulated by the APO E gene product [17]. However, recent evidence demonstrated that e4 allelic possession did not increase the degree of post traumatic cerebral swelling in adult TBI [18] and fatal paediatric brain trauma [19].

Arterial hypertension in adults was reported with the presence of either an e4 or e2 allele [20–23], but other

studies including an adult brain trauma cohort failed to demonstrate any genetic effects on arterial blood pressure measurements [24–26]. Our observed difference in CPP insult is therefore unlikely to be caused by genetic modulation of blood pressure.

Our patients possessing the e4 allele with poor outcome had experienced a lesser burden of CPP insult than the non-e4 carriers, and the amount of CPP insult was at a level that should have conferred good recovery. Carriers of the e4 allele may be less tolerant of CPP insult and develop more cerebral ischaemic damage than children without this allele so that even with the small amount of CPP insult they suffered, which should have conferred good recovery, the e4 carriers had poor outcome. This interpretation would support the observed trend of an increased incidence of severe ischaemic brain damage among the e4 carriers from a recent report of a postmortem study evaluating brain specimens from 239 fatal cases of TBI aged between 2 months and 84 years [27], although the burden of secondary CPP insult suffered by these patients were unknown.

Cerebral ischaemia may be caused by an imbalance of cerebral metabolic demand and substrate delivery. Healthy young adults and patients with Alzheimer's disease carrying the e4 allele were reported to have a reduced cerebral blood flow to selected regions of the brain [28, 29], but it remains unknown whether a similar effect occurs in children. If the potential increased ischaemic vulnerability observed in the e4 carriers is caused by allelic-related reduced cerebral blood flow, it will be particularly

important to avoid low CPP in these patients after brain trauma.

Cerebral metabolic rate in severely brain-injured children was shown to be normal in 81%, and this subsequently fell between the first and third day following brain trauma [30]. One may therefore postulate that the e4 allele increases its carriers' cerebral metabolic demand after brain trauma, thereby increasing their susceptibility to cerebral ischaemia. To assess this postulate, continuous measurements of cerebral metabolism and cerebral substrate supply would be required.

Recent animal studies have suggested that brain trauma causes an increased neuro-inflammation in transgenic animals carrying the human e4 allele when compared with those with the e3 allele [31, 32]. Circulating IL-10 levels among the e4 carriers with coronary artery disease were found to be significantly lower than the non-e4 carriers [33], but it is unknown whether APO E genotypes affect the degree of neuro-inflammation in TBI patients. Further studies are required to ascertain whether this may explain our observation on outcome which appeared out of proportion to the allelic-related variations in CPP insult post brain trauma. Another possible explanation for this observation is that the APO E e4 allele adversely affects outcome through mechanisms independent of the amount of CPP insult.

Almost all previous reports in the literature have suggested that the adverse effect of the APO E gene on outcome was related to the possession of the e4 allele. However, despite transgenic animal data suggesting a potentially more superior neurological repair offered by the e3 allele [34], no study has investigated whether APO E allelic influence on neurological recovery may be related to the absence of the e4 allele. The e3 homozygotic children in our cohort experienced more CPP insult than those in the other allelic groups which, by conventional evidence on the positive correlation between CPP insult and poor outcome [3, 4, 14], more of the e3 homozygous would have been expected to have an unfavourable outcome. However, we found fewer e3 homozygotic patients among the brain-injured children with a poor outcome. Additionally, children possessing only the e3 allele with 'good recovery' in our cohort had achieved that despite having suffered nearly 26 times more CPP insult than non-e3 homozygous. This would suggest that the e3 homozygous potentially enjoys a protective effect from ischaemic insult. This benefit may be due to the lack of the e4 or e2 alleles and warrants further investigations in a larger cohort.

APO E genetic polymorphisms and brain trauma outcome

The proportion of patients with poor outcome was unexpectedly low in our cohort and was a third less than the projected figure from previous report [5]. Fewer

unfavourable outcomes following childhood brain trauma, although a welcome finding, makes our study insufficiently powered to assess whether there is any allelic influence on neurological recovery after childhood brain trauma. Despite these limitations, we demonstrated that unfavourable outcome was 2.6 times more common among the e4 carriers than the e3 homozygotic patients, although 58% of the cohort were e3 homozygous and 22% were e4 carriers, who suffered the least amount of CPP insult. This highlighted the need to further investigate APO E allelic influence on childhood brain trauma.

Overrepresentation of the e2 allele in active children with or without brain injury

The patients in the previously reported APO E brain trauma studies [5–7, 16] were either exclusively or predominantly adults with very few children included. Although the APO E allelic distribution ratios from all these cohorts [5, 6, 16] were reported to be similar to previously published population data, none have actually included appropriate population controls for their injured cohorts. To our knowledge, this is the first study to investigate the relationship between APO E genotypes and outcome after brain trauma in an exclusive child cohort which includes an appropriate same population control group.

Our control subjects comprised healthy active children who had sustained minor injuries from their normal life styles and were age- and sex-matched to the brain-injured group. They were chosen to ensure as similar a risk for sustaining brain injury as possible to allow accurate comparison of the APO E allelic distribution frequency which we found to be similar between the groups. Our decision to include a separate paediatric population control group was further justified by the finding of a significant e2 allelic overrepresentation among our participants when compared to that of a previously reported group of healthy adults who were born in Northeast Scotland [15]. This adult cohort was chosen for comparison because it represented the largest reported healthy Scottish adult cohort to have their APO E genotyped, and its allelic distribution ratio (0.08 for e2, 0.77 for e3, and 0.15 for e4) was similar to those described in other adult brain trauma cohorts [5, 6, 16] and general populations worldwide [15, 35, 36], including different regions of Scotland, Germany, Taiwan and the USA. Geographic variation of the APO E allelic distribution is therefore unlikely to explain our observed overrepresentation of the e2 allele.

The frequency of the e2 allele among a previously reported healthy Scottish newborn population [37] recruited from the same geographic area as our cohort was similar to the e2 allele frequency of the above adult reports rather than to our study. Thus, our participants, children with minor

injuries and critically ill children post-brain trauma, may represent a different population to brain-injured adults and the general (adult and neonatal) populations. Paediatric injuries tend to occur more frequently because of the level of activity among normal young children, whilst adult injuries (include the elderly population) may occur regardless of their activity levels. Further investigation is required to determine whether APO E e2 allele relates to a heightened physical activity level.

Conclusion

Our result suggests that after childhood brain trauma, carriers of the APO E e4 allele may tolerate CPP insult less well than those with other allelic possession. APO E e3 homozygotic patients may enjoy a relative protective effect, mitigating CPP insult.

Acknowledgement We are grateful to our medical and nursing colleagues in the Intensive Care Units at RHSC, Edinburgh, and Newcastle General Hospital for their support with this project. We thank J. Croft and G. Wilson who facilitated demographic data and buccal smear collection for patients recruited in Tyneside.

Reference

- Chambers IR, Treadwell L, Mendelow AD (2000) The cause and incidence of secondary insults in severely head-injured adults and children. *Br J Neurosurg* 14:424–431
- Jones PA, Andrews PJ, Easton VJ, Minns RA (2003) Traumatic brain injury in childhood: intensive care time series data and outcome. *Br J Neurosurg* 17:29–39
- Chesnut RM, Marshall LF, Klauber MR, Blunt BA, Baldwin N, Eisenberg HM, Jane JA, Marmarou A, Foulkes MA (1993) The role of secondary brain injury in determining outcome from severe head injury. *J Trauma* 34:216–222
- Miller JD, Becker DP (1982) Secondary insults to the injured brain. *J R Coll Surg Edinb* 27:292–298
- Teasdale GM, Nicoll JA, Murray G, Fiddes M (1997) Association of apolipoprotein E polymorphism with outcome after head injury. *Lancet* 350:1069–1071
- Alexander S, Kerr ME, Kim Y, Kamboh MI, Beers SR, Conley YP (2007) Apolipoprotein E4 allele presence and functional outcome after severe traumatic brain injury. *J Neurotrauma* 24:790–797
- Friedman G, Froom P, Sazbon L, Grinblatt I, Shochina M, Tsenter J, Babaey S, Yehuda B, Groswasser Z (1999) Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. *Neurology* 52:244–248
- Teasdale GM, Murray GD, Nicoll JA (2005) The association between APOE epsilon4, age and outcome after head injury: a prospective cohort study. *Brain* 128:2556–2561
- Colton CA, Needham LK, Brown C, Cook D, Rasheed K, Burke JR, Strittmatter WJ, Schmechel DE, Vitek MP (2004) APOE genotype-specific differences in human and mouse macrophage nitric oxide production. *J Neuroimmunol* 147:62–67
- Mazur-Kolecka B, Dickson D, Frackowiak J (2006) Induction of vascular amyloidosis-beta by oxidative stress depends on APOE genotype. *Neurobiol Aging* 27:804–814
- Aono M, Lee Y, Grant ER, Zivin RA, Pearlstein RD, Warner DS, Bennett ER, Laskowitz DT (2002) Apolipoprotein E protects against NMDA excitotoxicity. *Neurobiol Dis* 11:214–220
- Mattson MP (1997) Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol Rev* 77:1081–1132
- Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *HhaI*. *J Lipid Res* 31:545–548
- Chambers IR, Jones PA, Lo TY, Forsyth RJ, Fulton B, Andrews PJ, Mendelow AD, Minns RA (2006) Critical thresholds of intracranial pressure and cerebral perfusion pressure related to age in paediatric head injury. *J Neurol Neurosurg Psychiatry* 77:234–240
- Cumming AM, Robertson FW (1984) Polymorphism at the apolipoprotein-E locus in relation to risk of coronary disease. *Clin Genet* 25:310–313
- Chiang MF, Chang JG, Hu CJ (2003) Association between apolipoprotein E genotype and outcome of traumatic brain injury. *Acta Neurochir (Wien)* 145:649–653 discussion 53–4
- Lynch JR, Pineda JA, Morgan D, Zhang L, Warner DS, Benveniste H, Laskowitz DT (2002) Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. *Ann Neurol* 51:113–117
- McCarron MO, Hoffmann KL, DeLong DM, Gray L, Saunders AM, Alberts MJ (1999) Intracerebral hemorrhage outcome: apolipoprotein E genotype, hematoma, and edema volumes. *Neurology* 53:2176–2179
- Quinn TJ, Smith C, Murray L, Stewart J, Nicoll JA, Graham DI (2004) There is no evidence of an association in children and teenagers between the apolipoprotein E epsilon4 allele and post-traumatic brain swelling. *Neuropathol Appl Neurobiol* 30:569–575
- Rantala M, Savolainen MJ, Kervinen K, Kesaniemi YA (1997) Apolipoprotein E phenotype and diet-induced alteration in blood pressure. *Am J Clin Nutr* 65:543–550
- Uusitupa M, Sarkkinen E, Kervinen K, Kesaniemi YA (1994) Apolipoprotein E phenotype and blood pressure. *Lancet* 343:57
- Uusitupa MI, Karhunen L, Rissanen A, Franssila-Kallunki A, Niskanen L, Kervinen K, Kesaniemi YA (1996) Apolipoprotein E phenotype modifies metabolic and hemodynamic abnormalities related to central obesity in women. *Am J Clin Nutr* 64:131–136
- Coudere R, Mahieux F, Bailleul S, Fenelon G, Mary R, Fermanian J (1993) Prevalence of apolipoprotein E phenotypes in ischemic cerebrovascular disease. A case-control study. *Stroke* 24:661–664
- de Knijff P, Boomsma DI, Feskens EJ, Jespersen J, Johansen LG, Kluit C, Kromhout D, Havekes LM (1994) Apolipoprotein E phenotype and blood pressure. *Lancet* 343:1234–1235
- Eto M, Watanabe K, Makino I (1989) Increased frequencies of apolipoprotein epsilon 2 and epsilon 4 alleles in patients with ischemic heart disease. *Clin Genet* 36:183–188
- Liaquat I, Dunn LT, Nicoll JA, Teasdale GM, Norrie JD (2002) Effect of apolipoprotein E genotype on hematoma volume after trauma. *J Neurosurg* 96:90–96
- Smith C, Graham DI, Murray LS, Stewart J, Nicoll JA (2006) Association of APOE e4 and cerebrovascular pathology in traumatic brain injury. *J Neurol Neurosurg Psychiatry* 77:363–366
- van Dyck CH, Gelernter J, MacAvoy MG, Avery RA, Criden M, Okereke O, Varma P, Seibyl JP, Hoffer PB (1998) Absence of an apolipoprotein E epsilon4 allele is associated with increased parietal regional cerebral blood flow asymmetry in Alzheimer disease. *Arch Neurol* 55:1460–1466
- Scarmeas N, Habeck CG, Stern Y, Anderson KE (2003) APOE genotype and cerebral blood flow in healthy young individuals. *Jama* 290:1581–1582
- Sharples PM, Stuart AG, Matthews DS, Aynsley-Green A, Eyre JA (1995) Cerebral blood flow and metabolism in children with severe head injury. Part 1: relation to age, Glasgow coma score,

- outcome, intracranial pressure, and time after injury. *J Neurol Neurosurg Psychiatry* 58:145–152
31. Guo L, LaDu MJ, Van Eldik LJ (2004) A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity. *J Mol Neurosci* 23:205–212
32. Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, Warner DS, Laskowitz DT (2003) APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 278:48529–48533
33. Tziakas DN, Chalikias GK, Antonoglou CO, Veletza S, Tentes IK, Kortsaris AX, Hatseras DI, Kaski JC (2006) Apolipoprotein E genotype and circulating interleukin-10 levels in patients with stable and unstable coronary artery disease. *J Am Coll Cardiol* 48:2471–2481
34. Sabo T, Lomnitski L, Nyska A, Beni S, Maronpot RR, Shohami E, Roses AD, Michaelson DM (2000) Susceptibility of transgenic mice expressing human apolipoprotein E to closed head injury: the allele E3 is neuroprotective whereas E4 increases fatalities. *Neuroscience* 101:879–884
35. Davignon J, Gregg RE, Sing CF (1988) Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 8:1–21
36. Utermann G, Langenbeck U, Beisiegel U, Weber W (1980) Genetics of the apolipoprotein E system in man. *Am J Hum Genet* 32:339–347
37. Becher JC, Bell JE, McIntosh N, Keeling JW (2005) Distribution of apolipoprotein E alleles in a Scottish healthy newborn population. *Biol Neonate* 88:164–167

PAPER 7 (IN PRESS)

Jones PA, Chambers IR, Minns RA, *Lo TYM*, Myles LM, Steers AJW.

Are head injury guidelines changing the outcome of head injured children? A regional investigation.

Acta Neurochirurgica (Supplement). 2008; (In press).

Are Head Injury Guidelines changing the outcome of head injured children? A regional investigation.

P A JONES¹, I R CHAMBERS³, R A MINNS^{1,2}, TYM LO², LM MYLES², AJW STEERS².

¹. Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh, EH9 1UW, Scotland, UK

². Royal Hospital for Sick Children, Edinburgh, 9 Sciennes Road, Edinburgh, EH9 1LF, Scotland, UK

³. Regional Medical Physics Department, The James Cook University Hospital, Marton Road, Middlesbrough, TS4 3BW, England, UK

Corresponding Author:

Professor Robert A Minns, PhD MB BS FRCP FRCPCH

Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh, EH9 1UW, Scotland, UK

Phone: +44 131 536 0800, Fax: +44 131 536 0821, Robert.Minns@ed.ac.uk

SUMMARY

Paediatric Head Injury *management* guidelines have been published in recent years, by SIGN (2000), RCPCH (2001), NICE (June 2003), and jointly by Critical/Intensive Care Societies (C/ICS July 2003).

We investigated whether outcome of children's Head Injury (HI) and total burden of secondary CPP insult has changed i) annually; ii) before and after the introduction of any HI guidelines, and iii) following other service changes.

76 children (aged 1 -<15 years with severe HI) were admitted to the Edinburgh Regional Head Injury Service between 1989 and 2006, and dichotomised at various time points and compared in terms of: demographic factors, age-banded Pressure-Time Index (PTI)), and Glasgow Outcome Scale (GOS) score.

When dichotomised around the **SIGN guidelines**, the outcome profiles were different ($p=0.03$), with 6, 2, 5 & 23 having GOS1 (died), GOS3 (severely disabled), GOS 4 (moderately disabled) and GOS5 (good recovery) before, compared to 4, 4, 17 & 15 respectively post-2000, with especially more cases of GOS4 than GOS5 ($p=0.007$). After the introduction of the **C/ICS guidelines** there was significantly less mean CPP insult ($p=0.030$). This lower amount of CPP insult has yet to be reflected in significantly better outcomes although the trend was for fewer deaths and severe disabilities.

Key Words: Paediatric Head Injury, Secondary Brain Insult, Outcome, Guidelines

Introduction:

Secondary pathophysiological CPP insult has been consistently shown to be related to outcome after head injury in both adults (4,5,6,9,11,13,16) and children (8,3,2,7), and improved management would be expected to reduce 2ndry brain insult in both duration and intensity. Paediatric Head Injury *management* guidelines have been published in recent years, by the Scottish Intercollegiate Guidelines Network (SIGN; 2000) (15), the Royal College of Paediatrics and Child Health (RCPCH; 2001) (14), the NHS National Institute for Clinical Excellence (NICE; June 2003) (12), and jointly by the Society of Critical Care Medicine, the World Federation of Pediatric Intensive and Critical Care Societies, and the Paediatric intensive Care Society UK (C/ICS; July 2003) (1). We investigated whether outcome after children's Head Injury and total burden of secondary brain insult, (particularly CPP insult) has changed i) annually; ii) before and after the introduction of any HI guidelines, and iii) following other service changes.

Materials and Methods:

Seventy-six children (aged 1 -<15 years with severe HI) were admitted to the Edinburgh Regional Head Injury Service for adults and children between 1989-1996 (Western General Hospital), and between 2000-2006 to a new Paediatric ICU (Royal Hospital for Sick Children). Demographic (age, gender, cause of injury, GCS, ISS, Marshall CT score, pupil response etc.) and physiological data were stored from the ICU bedside monitors, collected prospectively and analysed.

Outcome at 6-months post injury was assigned from responses to a questionnaire sent to all parent/carers. The groups were dichotomised at various time points, and compared in terms of: (i) demographic factors, (ii) secondary brain insults including Intracranial Pressure (ICP) and Cerebral Perfusion Pressure (CPP) insults, and (iii) Glasgow Outcome Scale (GOS – paediatric modification) score. We used a previously developed age-banded Pressure-Time

Index (PTI) (10) to give a measure of the amount of ‘brain insult’ which occurred during the ICU management period.

The Pressure-Time Index is a two-dimensional cumulative measure combining intensity and duration of secondary insult found in both ICP and CPP, calculated from data recorded every minute from the bed-side monitors in the Intensive Care Unit, using the example formula below for the cPTI for CPP:

$$cPTI = \sum (CPP_{threshold} - CPP) \times t_{sample} \text{ mmHg.min, where cPTI is the Cumulative Pressure}$$

Time Index, and t is the time at which the data was sampled.

The data set was divided so that cases admitted before *any* of the mentioned guidelines were published, were compared with those of later years (i.e. division point immediately pre SIGN guidelines - 2000). Data was also analysed on an annual basis to look for trends over time, and finally, the data set was split by admission date before and after July 2003, when the treatment specific guidelines of the C/ICS became widely available.

The statistical package of SPSS® for Windows 14.0 (SPSS Inc. U.S.A), was used for the analysis.

Results:

Pre- and post – SIGN Guidelines

When dichotomised around the **SIGN guidelines**, the groups were comparable with no statistically significant differences between the demographic features (age, sex, cause of injury, GCS, ISS, ICP monitoring characteristics etc) or in primary brain injury. The outcomes however, were different (Chi Square 9.11, p=0.028), with 6, 2, 5 & 23 having GOS 1 (died), GOS 3 (severely disabled), GOS 4 (moderately disabled) and GOS 5 (good recovery) before, compared to 4, 4, 17 & 15 respectively post-2000. In particular, the change in relative positions of the GOS 4 and 5 outcomes was highly significant (Chi Sq= 7.99, p<0.007) (Figure 1)

Insert Figure 1 about here

There was a (non-significant) trend for the later years to have longer mean insult durations of ICP, hypertension, CPP, hypoxia, pyrexia, tachycardia and bradycardia, greater mean cPTI for ICP, and a significantly greater mean number of episodes of CPP insults ($p=0.005$). i.e. a less optimal trend.

Fluctuations Annually

When these head injury cases were analysed year-by-year, there were no overall significant differences found but a closer look at the referral pattern (Figure 2) indicated that a change had taken place, with more children being referred from tertiary centres from 2001 onwards. There were 17, 1, and 18 admitted from the hospital Accident and Emergency department, a GP, and from tertiary referral centre before 2000, compared to 6, 1, and 33 respectively after 2000 (Chi Square = 9.49, $p=0.009$).

Insert Figure 2 about here

The *PTI_{icp} and PTI_{cpp} by year* (see figure 3) for all 76 patients from 1989 to 2006, and the median amount of measured secondary insult (cPTI) per patient, independent of outcome, was similar.

Insert Figure 3 about here

Pre- and post C/ICS Guidelines

When dichotomised around the time point of July 2003, when the paediatric C/ICS Head Injury guidelines were published, the outcomes were 9, 5, 14 & 29, compared to 1, 1, 8 & 9, for GOS 1, 3, 4, and 5 respectively, for the pre- and post- July 2003 groups. While overall this was not a significant difference, there was a trend for fewer deaths and poor (GOS 3) outcomes

The mean cPTI for CPP was 10,000.53 mmHg.min (the product of duration and intensity) compared to 4,218.37 mmHg.min respectively when the cohort was split pre- and post- July 2003. Although this gave an unequal distribution of cases (57 vs. 19), there was still significantly less CPP insult overall ($p=0.030$) after the introduction of the more

management-oriented **C/ICS guidelines**, with a decrease of almost 60%. The mean cPTI for ICP was 35,186.95 mmHg.min after July 2003, having fallen from 58,355.07 mmHg.min., a decrease of about 40%. While this shows a trend in the desired direction, it was not significant.

Discussion:

Pre- and Post- SIGN Guidelines

One of the notable guidelines advocated in the SIGN guidelines publication was for the transfer of head injured patients to a Neurosurgical Centre. We showed there was a significant change in referral pattern, with almost twice as many cases coming from other hospitals, largely in the eastern half of Scotland. However, an unexpected finding was an increase in the burden of ICP insult, as measured by the cPTI. We speculate that this could have been due to more insult occurring before admission to our unit, or different treatment routines employed before patient transfer. The difference however, was not due to the time interval from injury to the instigation of Intracranial Pressure Monitoring in these two groups and the later group were actually monitored on average, slightly more speedily (17.9 hours, compared to 13.8 hours). The mean duration of monitoring once at the PICU was 86.6 hours compared to 92.0 hours, but again this was not statistically significant.

As there were no demographic factors or GCS differences, it is difficult to explain the subsequent increase in numbers achieving only a moderate (GOS 4) recovery, compared to GOS5 (good recovery). The same questionnaire was used throughout the whole study period, and the same personnel were responsible for assigning the GOS score at 6 months, so internal bias is unlikely. We explored the change in the outcome pattern of those with GOS4 and GOS 5 more closely, and found that after 2000, the mean cPTI for CPP increased 3 fold, with a 4 fold increase in mean cPTI ICP in the same period, while those with GOS5 outcome had a 10% and 17% reduction in cPTI CPP and ICP respectively.

Annual Evaluation

The amount of cPTI for both ICP and CPP on a year-to-year basis, independent of outcome, was found to be not significantly different for the year groups as a whole. There were however relatively small number of cases per year.

Unsurprisingly, those with the poorest outcome had the greatest burden of secondary brain insult, whether considered annually, before and after 2000, or before and after the July 2003 dividing point.

Pre-and Post- C/ICS Guidelines

After the publication of the more management directed head injury C/ICS guidelines in July 2003, and despite the declining prevalence of paediatric head injury cases there was significantly less secondary 'pressure' insult: cPTI, for CPP ($p = 0.030$) and a trend to less ICP. This was accompanied by a trend to fewer deaths and GOS3 outcomes.

Clearly guidelines may be implemented completely or partially and will require some time to show an effect. Additional time will also ensure larger study numbers, however the trend is for a definite improvement in outcome and less secondary brain insult which may reach significance in the future.

Reference List

1. Adelson PD, Bratton SL, Carney NA, Chesnut RM, Du Coudray HEM, Goldstein B, Kochanek PM, Miller HC, Partington MD, Selden NR, Warden CR, Wright DW (2003) Guidelines for the acute medical management of severe traumatic brain injury in infants children and adolescents. *Journal of Trauma-Injury Infection & Critical Care* 54(SUPPL. 6): S237-S239
2. Chambers IR, Kirkham FJ (15-12-2003) What is the optimal cerebral perfusion pressure in children suffering from traumatic coma?. *Neurosurgical Focus* 15(6): E3-
3. Chambers IR, Treadwell L, Mendelow AD (2000) The cause and incidence of secondary insults in severely head-injured adults and children. *British Journal of Neurosurgery* 14(5): 424-431
4. Chambers IR, Treadwell L, Mendelow AD (2001) Determination of threshold levels of cerebral perfusion pressure and intracranial pressure in severe head injury

by using receiver-operating characteristic curves: an observational study in 291 patients. *Journal of Neurosurgery* 94(3): 412-416

5. Huang SJ, Hong WC, Han YY, Chen YS, Wen CS, Tsai YS, Tu YK (2006) Clinical outcome of severe head injury using three different ICP and CPP protocol-driven therapies. *Journal of Clinical Neuroscience* 13(8): 818-822
6. Huang SJ, Hong WC, Han YY, Chen YS, Wen CS, Tsan YS, Tu YK (2007) Clinical outcome of severe head injury in different protocol-driven therapies. *Journal of Clinical Neuroscience* 14(5): 449-454
7. Jackson S, Piper IR, Wagstaff A, Souter M (2000) A study of the effects of using different cerebral perfusion pressure (CPP) thresholds to quantify CPP "secondary insults" in children. *Acta Neurochirurgica - Supplement* 76453-456
8. Jones PA, Andrews PJ, Easton VJ, Minns RA (2003) Traumatic brain injury in childhood: intensive care time series data and outcome. *British Journal of Neurosurgery* 1729-39
9. Jones PA, Andrews PJ, Midgley S, Anderson SI, Piper IR, Tocher JL, Housley AM, Corrie JA, Slattery J, Dearden NM (1994) Measuring the burden of secondary insults in head-injured patients during intensive care. *Journal of Neurosurgical Anesthesiology* 6(1): 4-14
10. Jones PA, Chambers IR, Lo TY, Andrews PJ, Chaudhry W, Clark A, Croft J, Forsyth R, Fulton B, Mendelow AD, Wilson G, Minns RA (2005) Quantification of secondary CPP insult severity in paediatric head injured patients using a pressure-time index. *Acta Neurochirurgica - Supplement* 9529-32
11. Marmarou A, Saad A, Aygok G, Rigsbee M (2005) Contribution of raised ICP and hypotension to CPP reduction in severe brain injury: correlation to outcome. *Acta Neurochirurgica Suppl* 95277-280
12. NHS National Institute for Clinical Excellence (NICE) (2003) Head Injury. Triage, assessment, investigation and early management of head injury in infants, children and adults. Clinical Guideline 4
13. Rosner MJ, Rosner SD, Johnson AH (1995) Cerebral perfusion pressure: Management protocol and clinical results. *Journal of Neurosurgery* 83(6): 949-962
14. Royal College of Paediatrics and Child Health (2001) Good Practice Guidelines. Early Management of Patients with a Head Injury.
15. Scottish Intercollegiate Guideline Network (SIGN) (2000) Early Management of Patients with a Head Injury. No 46
16. Young JS, Blow O, Turrentine F, Claridge JA, Schulman A (15-12-2003) Is there an upper limit of intracranial pressure in patients with severe head injury if cerebral perfusion pressure is maintained? *Neurosurgical Focus* 15(6): E2-

Figure Legends

Figure 1.

Outcome at 6 months post-injury, illustrating that there were more GOS4 outcomes in later years compared to earlier years of the study.

GOS= Glasgow Outcome Score, where GOS1 = dead, GOS3 = Severe Disability, GOS4 = Moderate Disability, and GOS5= Good Recovery. (Note there were no cases of GOS2 =vegetative state).

Figure 2

Referral Pattern year by year. Since 2001 the referral pattern has changed, with more children being admitted from peripheral hospitals.

Figure 3

Box Plots of the yearly distribution of secondary brain insult assessed by the cumulative Pressure Time Index (for both CPP and ICP), with all outcomes included in each year group.

Figure 1:

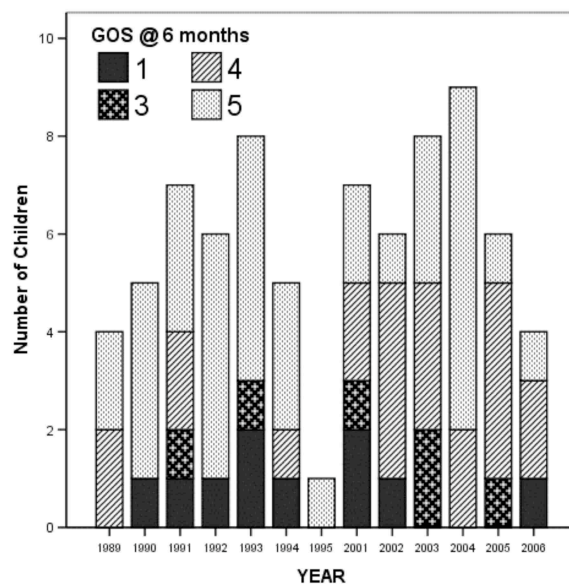


Figure 2:

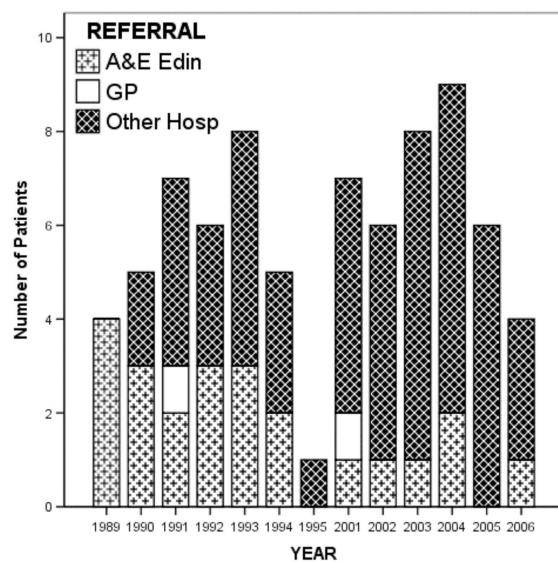
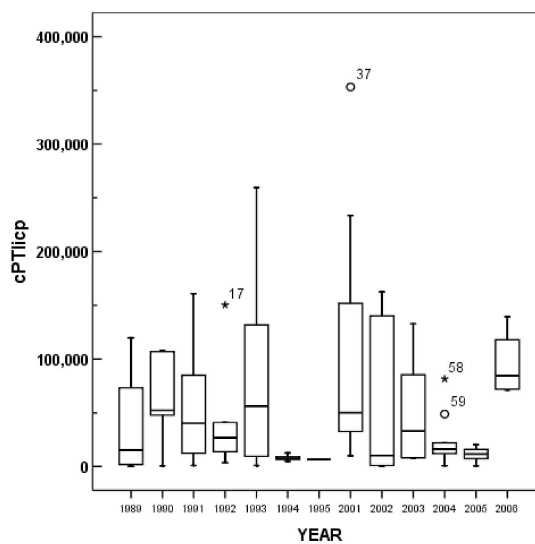
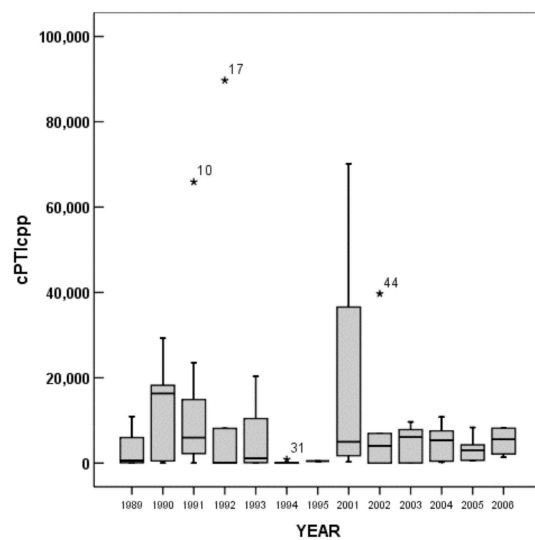


Figure 3:



PAPER 8 (IN PRESS)

Lo TYM, Jones PA, Minns RA.

Pediatric brain trauma outcome prediction using paired serum levels of inflammatory mediators and brain specific proteins.

***Journal of Neurotrauma.* 2009. (In press)**

Pediatric brain trauma outcome prediction using paired serum levels of inflammatory mediators and brain specific proteins

RUNNING TITLE: Paired biomarkers & brain trauma outcome prediction

TABLE OF CONTENT TITLE: Pediatric brain trauma outcome prediction using paired serum biomarkers

T.Y.M. Lo (MRCPCH);

P.A. Jones (M App Sci);

and R.A. Minns (FRCPCH)

Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh, EH9 1UW, UK

Corresponding Author

Dr. T.Y.M. Lo

Suite 2504, 218 Queens Quay West, Toronto, M5J 2Y6, Ontario, Canada

Telephone: +1 416 519 5739; Fax: +1 416 813 7299

Email: mils.lo@doctors.org.uk

Contact details for P.A. Jones and R.A. Minns:

Child Life and Health, University of Edinburgh, 20 Sylvan Place, Edinburgh, EH9 1UW, UK.

Telephone: +44 131 536 0805; Fax: +44 131 536 0822

Email: For P.A. Jones: Patricia.Jones@ed.ac.uk; For R.A. Minns: robert.minns@ed.ac.uk

Abstract:

Many potential brain trauma biomarkers have been reported but no previous study has described outcome prediction using combinations of biomarker levels. We aimed to investigate the outcome predictive values of multiple biomarkers from different mediator families and to determine whether combinations of 2 serum biomarkers may achieve higher outcome predictive values than individual biomarker levels. A prospective observational study was conducted involving 28 children requiring intensive care management following brain trauma. Day 1 post injury serum concentrations of 8 different biomarkers (S100b, NSE, IL-6, IL-8, IL-10, SICAM, L-selectin, and endothelin) were quantified using ELISA. Global outcome was assessed at 6 months post injury using the Glasgow Outcome Score (GOS). Receiver operator characteristic curve (ROC) analysis and its multivariate extension, Multivariate ROC (MultiROC), were used to assess the outcome predictive values of the individual and the paired biomarkers. None of the 8 biomarkers assessed individually achieved an area under the ROC curve (AUC) of more than 0.95 for predicting unfavourable outcome, but 5 of the 20 biomarker pairs assessed had this high degree of outcome predictability. Two combinations using S100b as the 'screening marker' and either L-selectin or IL-6 as the 'varying marker' achieved an AUC of 0.98, and their specificity and sensitivity for unfavourable outcome prediction were 96% and 100% respectively. Prognostic pairs combining serum levels of 2 biomarkers (inflammatory mediators and brain specific proteins) offer superior outcome predictive values for unfavourable outcome after childhood brain trauma than may be achieved using individual marker levels.

(Abstract word count: 245)

Key Words:

Pediatric traumatic brain injury

Brain specific proteins

Interleukins

Adhesion molecules

Outcome prediction

List of Biomarker Abbreviations:

IL-6 – Interleukin –6

IL-8 – Interleukin –8

IL-10 – Interleukin –10

S100b – S100b protein

SICAM – Soluble intracellular adhesion molecule

NSE – Neuron specific enolase

INTRODUCTION

Traumatic brain injury is the most common cause of death and disability in the pediatric population (Suominen, et al. 1998, Walker and Cass 1987). Accurate outcome prediction during the acute management of brain trauma remains difficult. Biochemical mediators are produced and released after brain trauma (Bell, et al. 1997, Ergun, et al. 1998, Fridriksson, et al. 2000, Kalabalikis, et al. 1999, Kossmann, et al. 1995, McClain, et al. 1991, McKeating, et al. 1998, Persson, et al. 1987, Pleines, et al. 1998, Raabe, et al. 1999, Raabe, et al. 1999, Skogseid, et al. 1992, Takahashi, et al. 1989, Whalen, et al. 1998, Woertgen, et al. 1997, Yamazaki, et al. 1995) to regulate the complex cascade of secondary brain insult and repair processes, or when there is neuronal or glial damage. Many of these mediators are measurable in blood or cerebrospinal fluid (CSF) making them potentially useful for outcome prediction (Bell, et al. 1997, Ergun, et al. 1998, Fridriksson, et al. 2000, Kalabalikis, et al. 1999, Kossmann, et al. 1995, McClain, et al. 1991, McKeating, et al. 1998, Persson, et al. 1987, Pleines, et al. 1998, Raabe, et al. 1999, Raabe, et al. 1999, Skogseid, et al. 1992, Takahashi, et al. 1989, Whalen, et al. 1998, Woertgen, et al. 1997, Yamazaki, et al. 1995).

The biochemical properties of potential brain trauma biomarkers previously described in the literature are diverse and include brain specific proteins such as S100b and NSE which represent the degree of astrocytic and neuronal damage (Ergun, et al. 1998, Marangos and Schmechel 1987, Rothoerl, et al. 1998, Skogseid, et al. 1992, Woertgen, et al. 1997, Yamazaki, et al. 1995); pro and anti-inflammatory

mediators such as IL-6 (Arand, et al. 2001, Chiaretti, et al. 2005, Minambres, et al. 2003) and IL-10 (Csuka, et al. 1999); chemotaxins such as IL-8 (Kossmann, et al. 1997, Kushi, et al. 2003, Whalen, et al. 2000); adhesion molecules such as L-selectin (McKeating, et al. 1998); and vasoconstrictors such as endothelin (Yang, et al. 2002). Most of these studies have only investigated the relationship between individual biomarker concentration and outcome without comparing the outcome predictive values of unrelated classes of biomarkers. It is, therefore, unclear from the existing literature whether inflammatory mediators or brain specific (neuronal or glial) proteins have higher outcome predictive values.

Translation of brain trauma biomarker research into clinically useful prognostic tools has not been successful. This may be because potentially useful prognostic thresholds have only been described in a few studies for a very limited number of selected biomarkers to predict unfavourable outcome (Ross, et al. 1996). In addition, no previous report in the literature has described outcome prediction using combinations of serum biomarker concentrations from different mediator families, which may offer more accurate outcome prediction than may be achieved with individual marker levels.

This study aims to determine the serum prognostic thresholds and predictive values of 8 biomarkers from different mediator families for unfavourable outcome after childhood isolated brain trauma. We additionally aim to determine which paired combinations of biomarkers are better at predicting unfavourable outcome after isolated pediatric brain trauma than individual marker levels.

MATERIALS AND METHODS

Patient Enrollment

Twenty-eight children (21 boys and 7 girls) consecutively admitted to neuro-intensive care after isolated accidental brain trauma were enrolled into a single-centre prospective observational study. The local ethics and hospital management committees approved the study. Parental consent was obtained for participation in the study.

Clinical and demographic details including age, sex, injury severity and initial brain CT findings were collected prospectively. Primary brain injury severity was assessed using the post-resuscitation/pre-intubation modified pediatric Glasgow Coma Scale (GCS) (Reilly, et al. 1988), and was dichotomised into ‘severe injury’ where GCS was 8 or less and ‘non-severe injury’ where GCS was more than 8 to assess their relationships with biomarker levels. Initial brain CT findings were dichotomised into ‘diffuse’ and ‘focal’ injuries. Global outcome was assessed at 6 months post injury using the modified Glasgow Outcome Score (GOS) (Adelson, et al. 1997) and dichotomised into ‘favourable recovery’ when GOS 4 and 5 were achieved, and ‘unfavourable outcome’ when GOS were between 1 and 3.

Sample Collection and Measurement

All patients had an arterial blood sample collected at precisely 24 hours (day 1) post injury. The blood samples were collected in pyrogen-free plastic tubes and centrifuged at 1200 g for 10 minutes. The serum was removed immediately and stored in pyrogen-free plastic tubes at -70°C until analyses.

Commercially available enzyme linked immunoassays (ELISA) were used to quantify the serum concentrations of 8 different biomarkers (Table 1). Samples were analysed in duplicate and averaged to provide the final marker concentrations.

Outcome Predictive Values and Prognostic Thresholds of Individual Biomarkers

Mann Whitney U tests were employed to detect statistical difference ($p < 0.05$) in mediator concentrations between the dichotomised injury severity and outcome groups. For each biomarker with significantly different levels between the outcome dichotomy, sensitivity and specificity of incremental values for prediction of unfavourable outcome at 6 months post brain injury were calculated. Receiver Operator Characteristic (ROC) curves were then plotted and the optimal cut-off value (prognostic threshold) for each mediator, which was defined as the point closest to the left upper corner of the ROC curve, was identified. Areas under the ROC curves (AUC) were measured to compare the outcome predictive values between individual biomarkers.

Paired Biomarker Levels and Multivariate ROC Curves

The algorithm of paired biomarkers consisted of a Boolean expression of the mediator concentrations related by an algebraic operator as the components of the expression (i.e. biomarker A > a predefined 'screening' threshold AND biomarker B > incremental thresholds predicting unfavourable outcome). One of the paired biomarkers was called the 'screening marker' and had a single cut-point ('screening threshold') assigned. The other biomarker ('varying marker') had incremental thresholds, and corresponding sensitivities and specificities were calculated to plot the multivariate ROC (MultiROC) curve. Area under each MultiROC curve (AUC_m) was measured to compare the outcome prediction performance between the different paired combinations. AUC_m of the pairs were then compared with the AUC of the individual biomarkers to determine whether they or individual biomarkers had higher outcome predictive values.

The 'screening threshold' of each biomarker was determined by plotting individual mediator concentrations against the outcome dichotomy and was defined as the highest biomarker level that identified most patients with favourable recovery without including any patient with unfavourable outcome. In order to investigate the effect of different 'screening threshold' levels within each combination and whether these might better differentiate outcome, the original 'screening threshold' within each pair was reduced first by 10%, then 20%, and MultiROC curves were plotted again with the new 'screening threshold' test levels, and the AUC_m were determined. The analyses were repeated when the original 'screening thresholds' were increased by 10% and 20%.

The optimal threshold for the ‘varying marker’ of each combination was determined, which was defined as the point closest to the left upper corner of the MultiROC curve. Biomarkers that individually did not predict outcome were excluded from all MultiROC analyses.

RESULTS

Demographics and Outcome

Table 2 summarises the demographic details. The median GCS was 7 (range 3 to 13). Median age of the patients was 8.59 years (range 0.33 to 14.17 years). At 6 months post injury, 24 patients made a favourable recovery while 4 children had an unfavourable outcome.

Biomarker Concentrations and Demographic Dichotomies

Of the 8 biomarkers assessed, only the SICAM and IL-6 levels were significantly higher in patients with severe injury than those with non-severe injury ($p = 0.01$ for SICAM, and $p = 0.02$ for IL-6). The median NSE serum levels in patients with diffuse brain injury (identified on the initial brain CT scan) were two times higher than those with focal injury ($p = 0.01$). None of the other biomarker levels differentiated diffuse and focal injuries.

Individual Neurochemical Mediator Concentrations and Outcome

Patients with unfavourable outcome had significantly higher day 1 serum concentrations of S-100b, NSE, L-selectin, IL-6, and IL-8 than those with favourable recovery (Figure 1). The outliers in the S100b and IL-8 figures did not have any clinical, physiological or other apparent differences to explain their outlying neurochemical levels. The outliers in both of these figures (S100b and IL-8) were different patients. Day 1 L-selectin level had the highest predictive value for unfavourable outcome at 6 months after brain trauma (AUC = 0.92). Table 3 summarises the area under the ROC curves for individual biomarkers, their prognostic thresholds (optimal cut-point), and the corresponding sensitivities and specificities.

Paired Biomarker Levels and Outcome Prediction

Because IL-10, endothelin, and SICAM levels did not differentiate between the outcome dichotomies, they were excluded from MultiROC analyses. There were, therefore, 5 possible screening markers. ‘Screening threshold’ for each ‘screening marker’ is shown in Figure 1. Each ‘screening marker’ was paired in turn with the remaining 4 biomarkers to form 20 different combinations. Table 4 summarises the top 10 combinations for unfavourable outcome prediction after childhood brain trauma, their AUCm, and optimal threshold for the ‘varying marker’ within each of these combinations. Two pairs achieved 100% sensitivity and 96% specificity for unfavourable outcome prediction (Table 4).

Comparison of the Predictability for Unfavourable Outcome after Brain Trauma

When using individual biomarker level to predict unfavourable outcome, only the day 1 L-selectin level had an AUC > 0.90 (Table 3). 15 of the 20 combinations examined had AUC > 0.90 for prediction of unfavourable outcome after brain trauma. Combinations using S100b as the 'screening marker' had higher predictive values for unfavourable outcome than those using other mediators as the 'screening marker'. Outcome predictive values were higher in combinations that used L-selectin as the 'varying marker' than those using it as the 'screening marker'. Increasing or decreasing the 'screening threshold' of each combination did not improve prediction for unfavourable outcome (Table 5).

A day 1 L-Selectin level of 1200ng/ml was 75% sensitive and 88% specific for predicting unfavourable outcome after brain trauma, but the specificity increased to 96% when this threshold was coupled with either S100b or NSE as the 'screening marker' (Figure 2).

DISCUSSION

This paediatric brain trauma study demonstrated that the day 1 serum concentrations of inflammatory mediators had higher prognostic values than brain specific proteins, but the best outcome predictive value was achieved with combinations of 2 biomarker levels from different mediator families.

Serum or CSF levels of brain specific proteins and inflammatory mediators such as S100b, NSE, IL-6, and IL-8 measured at various time points after brain trauma have individually been described in the literature as potentially useful predictors of unfavourable outcome (Arand, et al. 2001, Bandyopadhyay, et al. 2005, Chiaretti, et al. 2005, Jackson, et al. 2000, Kushi, et al. 2003, Minambres, et al. 2003, Ross, et al. 1996, Rothoerl, et al. 1998, Spinella, et al. 2003). It has not, however, been possible to ascertain from the existing literature which class and specific biomarker offers the most accurate outcome prediction after brain trauma. This may be because the majority of previous studies have concentrated on evaluating a single mediator or limited numbers of mediators from the same biomarker family. Additionally, reports in the literature often use different specimen types (CSF, arterial or venous blood), sample collection time-points, and data analyses, making comparison between studies difficult.

Buttram and colleagues recently used multiplex bead array to quantify the CSF levels of 21 different cytokines at 4 various time points after pediatric brain trauma but failed to demonstrate any associations with outcome (Buttram, et al. 2007). Their cohort comprised of 36 infants and children who had diverse mechanisms of injury which included isolated traumatic brain injury, inflicted brain trauma, and polytrauma with widely variable time ranges for each sample collection point (Buttram, et al. 2007). Our study, in contrast, restricted evaluations to serum biomarker levels measured at exactly 24 hours and only in isolated accidental brain trauma. This may explain the difference in outcome prediction of biomarkers

between both studies, and the high predictive values for unfavourable outcome observed in our study. A recent study has demonstrated that the time courses of serum brain specific protein levels differ significantly between different types of pediatric brain injury (Berger, et al. 2006).

Our study also evaluated biomarkers from different mediator families and demonstrated for the first time that some inflammatory markers (L-selectin and IL-8) had higher predictive values for unfavourable outcome than brain specific (glial or neuronal) proteins. One possible explanation for our findings is that although serum levels of proteins released from glia or neurons after brain injury may not accurately reflect the extent of cellular damage. Serum inflammatory marker levels may, on the other hand, have better associations with the actual burden of neuro-inflammation which in turn has a higher outcome predictive value. Alternatively, neuro-inflammation may have a greater influence on outcome after brain trauma than previously expected.

We demonstrated for the first time that the outcome predictive values vary between inflammatory mediators with L-selectin offering a higher predictive value than IL-6 and IL-8, while IL-10 and SICAM serum levels had no association with outcome. Neuro-inflammation is a heterogeneous process with different pathways operating and subsiding at various time-points after brain trauma. To investigate this complex relationship between different biomarkers, their different time-courses and outcome prediction will require a larger cohort with sufficient numbers of patients with poor outcome. This may be difficult to achieve in the light of the Western world-wide

declining incidence of childhood brain trauma (Engberg and Teasdale 1998, Ponsky, et al. 2005, Thurman and Guerrero 1999), and improvement in brain trauma management and outcome.

Increasing numbers of biomarkers have been described as potentially useful prognosticators of brain trauma outcome, but none have actually been translated into clinically useful entities. This may be because the majority of papers in the literature have only reported on the varying concentrations of the different biomarkers in relation to outcome and only a few prior studies have proposed potentially useful prognostic thresholds, such as NSE (Ross, et al. 1996). Our study is the first to describe serum prognostic thresholds for unfavourable outcome in 5 biomarkers simultaneously and includes some of the previously less well investigated biomarkers such as L-selectin.

Previous studies using individual biomarkers rarely yielded sensitivity and specificity of more than 85% (as reported here) for unfavourable outcome prediction (Ross, et al. 1996). Brain trauma outcome is dependent upon many factors such as the severity of the primary brain injury (Bruce, et al. 1978, Facco, et al. 1986, Pal, et al. 1989, Prasad, et al. 2002) and the burden of secondary insults (Chambers, et al. 2006, Chambers, et al. 2000, Chesnut, et al. 1993, Jones, et al. 2003, Miller and Becker 1982), and to expect successful and accurate prognostication with a single serum biomarker is unrealistic. Bergers and colleagues investigated the outcome predictive values of the serum levels of 3 brain specific proteins (S100b, NSE and myelin basic protein) in 152 children with brain trauma (Berger, et al. 2007). Using

binary logistic regression to evaluate the simultaneous effects of these 3 biomarkers on outcome, they described a 77% correct classification rate and a positive predictive value of 75%. However, no biomarker prognostic threshold was described in their report (Berger, et al. 2007). In 2 recent independent reports, Bergers and colleagues described the usefulness of combined serum biomarker levels to respectively diagnose pediatric brain trauma (Berger, et al. 2005), and inflicted brain injury among well infants with GCS of 15 (Berger, et al. 2008). Neither of these studies investigated outcome predictive values using combinations of biomarkers (Berger, et al. 2005, Berger, et al. 2008). Our study is, therefore, the first to report brain trauma outcome prediction using biomarkers from different mediator families, and to describe prognostic thresholds for several paired biomarker combinations.

We used multivariate ROC (MultiROC) curves for our analyses because they retain all the simplicity of interpretations of the traditional ROC curve analysis but additionally allow comparisons between the performance of multivariate combinations without being restricted to the display of a single variable's performance and comparisons of individual tests (Shultz 1995). Our chosen 'screening thresholds' were based upon the highest concentration of each biomarker that identified as many patients with favourable recovery without including any of those with unfavourable outcome. We did not know at the outset of the study whether these thresholds would offer the highest predictive values but have subsequently demonstrated that arbitrary increasing or decreasing the original threshold levels by 10% and 20% did not improve the outcome predictive values of these combinations. Using the MultiROC curves, we further refined our prognostic

algorithm by defining the optimal threshold for the ‘varying biomarker’ within each combination, and were able to propose for the first time pairs of biomarker levels that were highly predictive of unfavourable outcome.

Combinations using a brain specific protein (S100b or NSE) as the ‘screening marker’ had higher predictive values for unfavourable outcome than those that used either of these proteins as the ‘varying marker’. On the other hand, L-selectin offered a higher outcome predictive value when it was used as the ‘varying marker’ rather than the ‘screening marker’ within the prognostic algorithm. One possible explanation for these observations was that the ‘screening marker’ thresholds we chose for S100b or NSE were more sensitive in screening for patients with unfavourable outcome than that chosen for L-selectin. Alternatively, although elevated brain specific proteins levels signalled glial and neuronal damage, their relatively short serum half life meant that their serum levels at the time of sampling in our cohort might have been better employed as a screening tool for unfavourable outcome.

The main limitation of our study is relatively small cohort size and the few patients with unfavourable outcome resulting in the need to assess outcome simply, rather than using more elegant measures such as detailed cognitive tests (Beers, et al. 2007). Another limitation is that our findings may not be used to predict outcome of brain trauma patients with extra-cranial injuries who may have greatly altered serum cytokine profiles independent of the brain injury because our cohort only includes patients with isolated traumatic brain injuries. Despite these limitations, we have

successfully defined prognostic thresholds for 5 individual biomarkers and combinations of paired biomarker levels with very high outcome predictive values. Our proposed prognostic thresholds and their outcome predictive values were not validated because of the small sample size. Our findings may, however, be used as starting points for validation in future studies involving larger and independent cohorts before translation into potentially useful clinical tools for brain trauma outcome predictions.

CONCLUSION

Combining brain specific protein and inflammatory mediator levels offer higher outcome predictive values than may be achieved with the individual biomarkers.

(Manuscript word count: 3088)

AUTHOR DISCLOSURE STATEMENT

The authors declare that there is no potential conflict of interest.

REFERENCE

Adelson, P.D., Clyde, B., Kochanek, P.M., Wisniewski, S.R., Marion, D.W., Yonas, H. (1997). Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. *Pediatr Neurosurg* 26, 200-207.

Arand, M., Melzner, H., Kinzl, L., Bruckner, U.B., Gebhard, F. (2001). Early inflammatory mediator response following isolated traumatic brain injury and other major trauma in humans. *Langenbecks Arch Surg* 386, 241-248.

Bandyopadhyay, S., Hennes, H., Gorelick, M.H., Wells, R.G., Walsh-Kelly, C.M. (2005). Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. *Acad Emerg Med* 12, 732-738.

Beers, S.R., Berger, R.P., Adelson, P.D. (2007). Neurocognitive outcome and serum biomarkers in inflicted versus non-inflicted traumatic brain injury in young children. *J Neurotrauma* 24, 97-105.

Bell, M.J., Kochanek, P.M., Doughty, L.A., Carcillo, J.A., Adelson, P.D., Clark, R.S., Wisniewski, S.R., Whalen, M.J., DeKosky, S.T. (1997). Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma* 14, 451-457.

Berger, R.P., Adelson, P.D., Pierce, M.C., Dulani, T., Cassidy, L.D., Kochanek, P.M. (2005). Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. *J Neurosurg* 103, 61-68.

Berger, R.P., Adelson, P.D., Richichi, R., Kochanek, P.M. (2006). Serum biomarkers after traumatic and hypoxemic brain injuries: insight into the biochemical response of the pediatric brain to inflicted brain injury. *Dev Neurosci* 28, 327-335.

Berger, R.P., Beers, S.R., Richichi, R., Wiesman, D., Adelson, P.D. (2007). Serum biomarker concentrations and outcome after pediatric traumatic brain injury. *J Neurotrauma* 24, 1793-1801.

Berger, R.P., Ta'asan, S., Rand, A., Lokshin, A., Kochanek, P. (2008). Multiplex assessment of serum biomarker concentrations in well-appearing children with inflicted traumatic brain injury. *Pediatr Res*.

Bruce, D.A., Schut, L., Bruno, L.A., Wood, J.H., Sutton, L.N. (1978). Outcome following severe head injuries in children. *J Neurosurg* 48, 679-688.

Buttram, S.D., Wisniewski, S.R., Jackson, E.K., Adelson, P.D., Feldman, K., Bayir, H., Berger, R.P., Clark, R.S., Kochanek, P.M. (2007). Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. *J Neurotrauma* 24, 1707-1717.

Chambers, I.R., Jones, P.A., Lo, T.Y., Forsyth, R.J., Fulton, B., Andrews, P.J., Mendelow, A.D., Minns, R.A. (2006). Critical thresholds of intracranial pressure and

cerebral perfusion pressure related to age in paediatric head injury. *J Neurol Neurosurg Psychiatry* 77, 234-240.

Chambers, I.R., Treadwell, L., Mendelow, A.D. (2000). The cause and incidence of secondary insults in severely head-injured adults and children. *Br J Neurosurg* 14, 424-431.

Chesnut, R.M., Marshall, L.F., Klauber, M.R., Blunt, B.A., Baldwin, N., Eisenberg, H.M., Jane, J.A., Marmarou, A., Foulkes, M.A. (1993). The role of secondary brain injury in determining outcome from severe head injury. *J Trauma* 34, 216-222.

Chiaretti, A., Genovese, O., Aloe, L., Antonelli, A., Piastra, M., Polidori, G., Di Rocco, C. (2005). Interleukin 1beta and interleukin 6 relationship with paediatric head trauma severity and outcome. *Childs Nerv Syst* 21, 185-193; discussion 194.

Csuka, E., Morganti-Kossmann, M.C., Lenzlinger, P.M., Joller, H., Trentz, O., Kossmann, T. (1999). IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol* 101, 211-221.

Engberg, A., Teasdale, T.W. (1998). Traumatic brain injury in children in Denmark: a national 15-year study. *Eur J Epidemiol* 14, 165-173.

Ergun, R., Bostanci, U., Akdemir, G., Beskonakli, E., Kaptanoglu, E., Gursoy, F., Taskin, Y. (1998). Prognostic value of serum neuron-specific enolase levels after head injury. *Neurol Res* 20, 418-420.

Facco, E., Zuccarello, M., Pittoni, G., Zanardi, L., Chiaranda, M., Davia, G., Giron, G.P. (1986). Early outcome prediction in severe head injury: comparison between children and adults. *Childs Nerv Syst* 2, 67-71.

Fridriksson, T., Kini, N., Walsh-Kelly, C., Hennes, H. (2000). Serum neuron-specific enolase as a predictor of intracranial lesions in children with head trauma: a pilot study. *Acad Emerg Med* 7, 816-820.

Jackson, R.G., Samra, G.S., Radcliffe, J., Clark, G.H., Price, C.P. (2000). The early fall in levels of S-100 beta in traumatic brain injury. *Clin Chem Lab Med* 38, 1165-1167.

Jones, P.A., Andrews, P.J., Easton, V.J., Minns, R.A. (2003). Traumatic brain injury in childhood: intensive care time series data and outcome. *Br J Neurosurg* 17, 29-39.

Kalabalikis, P., Papazoglou, K., Gouriots, D., Papadopoulos, N., Kardara, M., Papageorgiou, F., Papadatos, J. (1999). Correlation between serum IL-6 and CRP levels and severity of head injury in children. *Intensive Care Med* 25, 288-292.

Kossmann, T., Hans, V.H., Imhof, H.G., Stocker, R., Grob, P., Trentz, O., Morganti-Kossmann, C. (1995). Intrathecal and serum interleukin-6 and the acute-phase response in patients with severe traumatic brain injuries. *Shock* 4, 311-317.

Kossmann, T., Stahel, P.F., Lenzlinger, P.M., Redl, H., Dubs, R.W., Trentz, O., Schlag, G., Morganti-Kossmann, M.C. (1997). Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J Cereb Blood Flow Metab* 17, 280-289.

Kushi, H., Saito, T., Makino, K., Hayashi, N. (2003). IL-8 is a key mediator of neuroinflammation in severe traumatic brain injuries. *Acta Neurochir Suppl* 86, 347-350.

Marangos, P.J., Schmechel, D.E. (1987). Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. *Annu Rev Neurosci* 10, 269-295.

McClain, C., Cohen, D., Phillips, R., Ott, L., Young, B. (1991). Increased plasma and ventricular fluid interleukin-6 levels in patients with head injury. *J Lab Clin Med* 118, 225-231.

McKeating, E.G., Andrews, P.J., Mascia, L. (1998). The relationship of soluble adhesion molecule concentrations in systemic and jugular venous serum to injury severity and outcome after traumatic brain injury. *Anesth Analg* 86, 759-765.

Miller, J.D., Becker, D.P. (1982). Secondary insults to the injured brain. *J R Coll Surg Edinb* 27, 292-298.

Minambres, E., Cemborain, A., Sanchez-Velasco, P., Gandarillas, M., Diaz-Reganon, G., Sanchez-Gonzalez, U., Leyva-Cobian, F. (2003). Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. *Crit Care Med* 31, 933-938.

Pal, J., Brown, R., Fleischer, D. (1989). The value of the Glasgow Coma Scale and Injury Severity Score: predicting outcome in multiple trauma patients with head injury. *J Trauma* 29, 746-748.

Persson, L., Hardemark, H.G., Gustafsson, J., Rundstrom, G., Mendel-Hartvig, I., Esscher, T., Pahlman, S. (1987). S-100 protein and neuron-specific enolase in cerebrospinal fluid and serum: markers of cell damage in human central nervous system. *Stroke* 18, 911-918.

Pleines, U.E., Stover, J.F., Kossmann, T., Trentz, O., Morganti-Kossmann, M.C. (1998). Soluble ICAM-1 in CSF coincides with the extent of cerebral damage in patients with severe traumatic brain injury. *J Neurotrauma* 15, 399-409.

Ponsky, T.A., Eichelberger, M.R., Cardozo, E., Huang, Z.J., Pratsch, G.L., Thuma-Croom, S.E., Newman, K.D. (2005). Analysis of head injury admission trends in an urban American pediatric trauma center. *J Trauma* 59, 1292-1297.

Prasad, M.R., Ewing-Cobbs, L., Swank, P.R., Kramer, L. (2002). Predictors of outcome following traumatic brain injury in young children. *Pediatr Neurosurg* 36, 64-74.

Raabe, A., Grolms, C., Seifert, V. (1999). Serum markers of brain damage and outcome prediction in patients after severe head injury. *Br J Neurosurg* 13, 56-59.

Raabe, A., Grolms, C., Sorge, O., Zimmermann, M., Seifert, V. (1999). Serum S-100B protein in severe head injury. *Neurosurgery* 45, 477-483.

Reilly, P.L., Simpson, D.A., Sprod, R., Thomas, L. (1988). Assessing the conscious level in infants and young children: a paediatric version of the Glasgow Coma Scale. *Childs Nerv Syst* 4, 30-33.

Ross, S.A., Cunningham, R.T., Johnston, C.F., Rowlands, B.J. (1996). Neuron-specific enolase as an aid to outcome prediction in head injury. *Br J Neurosurg* 10, 471-476.

Rothoerl, R.D., Woertgen, C., Holzsuh, M., Metz, C., Brawanski, A. (1998). S-100 serum levels after minor and major head injury. *J Trauma* 45, 765-767.

Shultz, E.K. (1995). Multivariate receiver-operating characteristic curve analysis: prostate cancer screening as an example. *Clin Chem* 41, 1248-1255.

Skogseid, I.M., Nordby, H.K., Urdal, P., Paus, E., Lilleaas, F. (1992). Increased serum creatine kinase BB and neuron specific enolase following head injury indicates brain damage. *Acta Neurochir (Wien)* 115, 106-111.

Spinella, P.C., Dominguez, T., Drott, H.R., Huh, J., McCormick, L., Rajendra, A., Argon, J., McIntosh, T., Helfaer, M. (2003). S-100beta protein-serum levels in healthy children and its association with outcome in pediatric traumatic brain injury. *Crit Care Med* 31, 939-945.

Suominen, P., Kivioja, A., Ohman, J., Korpela, R., Rintala, R., Olkkola, K.T. (1998). Severe and fatal childhood trauma. *Injury* 29, 425-430.

Takahashi, H., Sato, H., Tsuji, Y. (1989). Biochemical markers in the acute stage of head injury--aldosterone and CK-BB. *Neurol Med Chir (Tokyo)* 29, 192-195.

Thurman, D., Guerrero, J. (1999). Trends in hospitalization associated with traumatic brain injury. *Jama* 282, 954-957.

Walker, P.J., Cass, D.T. (1987). Paediatric trauma: urban epidemiology and an analysis of methods for assessing the severity of trauma in 598 injured children. *Aust N Z J Surg* 57, 715-722.

Whalen, M.J., Carlos, T.M., Kochanek, P.M., Wisniewski, S.R., Bell, M.J., Carcillo, J.A., Clark, R.S., DeKosky, S.T., Adelson, P.D. (1998). Soluble adhesion molecules in CSF are increased in children with severe head injury. *J Neurotrauma* 15, 777-787.

Whalen, M.J., Carlos, T.M., Kochanek, P.M., Wisniewski, S.R., Bell, M.J., Clark, R.S., DeKosky, S.T., Marion, D.W., Adelson, P.D. (2000). Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. *Crit Care Med* 28, 929-934.

Woertgen, C., Rothoerl, R.D., Holzschuh, M., Metz, C., Brawanski, A. (1997). Comparison of serial S-100 and NSE serum measurements after severe head injury. *Acta Neurochir (Wien)* 139, 1161-1164; discussion 1165.

Yamazaki, Y., Yada, K., Morii, S., Kitahara, T., Ohwada, T. (1995). Diagnostic significance of serum neuron-specific enolase and myelin basic protein assay in patients with acute head injury. *Surg Neurol* 43, 267-270; discussion 270-261.

Yang, Y., Huang, W., Lu, X. (2002). Early changes of endothelin, nitric oxide and arginine-vasopressin in patients with acute cerebral injury. *Chin J Traumatol* 5, 259-262.

Table 1: Commercially available ELISA used in the study

<p>Brain Specific Proteins:</p> <p>Glial Protein:</p> <ul style="list-style-type: none">○ S-100b (Nexus Dx™ S-100 Test Kit, Synx Pharma Inc) <p>Neuronal Protein:</p> <ul style="list-style-type: none">○ NSE (Nexus Dx™ NSE Test Kit, Synx Pharma Inc)
<p>Inflammatory Mediators:</p> <p>Pro-inflammatory mediator:</p> <ul style="list-style-type: none">○ IL-6 (IL-6 ELISA Kit, Diaclone Research) <p>Chemotaxin:</p> <ul style="list-style-type: none">○ IL-8 (IL-8 ELISA kit, Diaclone Research) <p>Anti-inflammatory mediator:</p> <ul style="list-style-type: none">○ IL-10 (IL-10 ELISA Kit, Diaclone Research) <p>Adhesion Molecules:</p> <ul style="list-style-type: none">○ L-selectin (human sL-Selectin Immunoassay, R&D Systems)○ SICAM (sICAM-1 ELISA Kit, Diaclone Research)
<p>Vasoconstrictor:</p> <ul style="list-style-type: none">○ Endothelin (Endothelin 1-21 Test Kit, Biomedica)

Table 1 Legend:

Table 1 summarises the commercially available ELISA used in the study.

Table 2: Demographic details and outcome of the cohort

	Favourable Outcome	Unfavourable Outcome
Median age	7.92 (Range 0.33 – 14.17 yrs)	10.50 years (Range 2.33 – 13.42 yrs)
Sex distribution:		
Boys	19	2
Girls	5	2
Post-resuscitation GCS (Primary brain injury severity):		
GCS 13 – 15 (Non-severe)	2	0
GCS 9 – 12 (Non-severe)	8	1
GCS 3 – 8 (severe)	14	3
Initial CT brain findings:		
Diffuse injury	15	4
Focal injury	9	0

Table 2 Legend:

Table 2 summarises the demographic details and outcome of the cohort.

Table 3: The optimal cut-off value of individual biomarkers and their sensitivity and specificity for unfavourable outcome prediction.

Prediction of Unfavourable Outcome at 6 Months Post Injury				
	Optimal Cut-off Value on ROC	AUC	Specificity	Sensitivity
L-selectin Day 1 Level	1200 ng/ml	0.92	88%	75%
IL-8 Day 1 Level	30 pg/ml	0.88	92%	75%
NSE Day 1 Level	25 ng/ml	0.83	83%	75%
S100B Day 1 Level	0.05 ng/ml	0.83	79%	75%
IL-6 Day 1 Level	60 pg/ml	0.83	71%	75%

Table 3 Legend:

Table 3 summarises the optimal cut-off value of individual biomarkers and their sensitivity and specificity for unfavourable outcome prediction.

Table 4: Top 10 paired biomarker levels for unfavourable outcome prediction, the optimal prognostic threshold for their ‘varying marker’, AUCm, specificity and sensitivity.

Prediction of Unfavourable Outcome at 6 Months Post Injury				
Paired Biomarkers (‘Screening marker > ‘Screening threshold’ AND ‘Varying marker’)	Optimal Cut-off Value for the ‘Varying Marker’ on MutliROC	AUCm (Area under the MultiROC)	Specificity	Sensitivity
S100b > 0.04 ng/ml AND L-selectin	1000 ng/ml	0.98	96%	100%
S100b > 0.04 ng/ml AND IL-6	40 pg/ml	0.98	96%	100%
S100b > 0.04 ng/ml AND NSE	10 ng/ml	0.97	92%	100%
IL-6 > 30 pg/ml AND L-selectin	1000 ng/ml	0.96	88%	100%
NSE > 12 ng/ml AND L-selectin	1200 ng/ml	0.95	96%	75%
NSE > 12 ng/ml AND S100b	0.05 ng/ml	0.94	92%	75%
IL-6 > 30 pg/ml AND IL-8	30 pg/ml	0.93	96%	75%
S100b > 0.04 ng/ml AND IL-8	30 pg/ml	0.93	92%	75%
IL-8 > 20 pg/ml AND L-selectin	1200 ng/ml	0.92	88%	75%
L-selectin > 700 ng/ml AND IL-8	30 pg/ml	0.92	96%	75%

Table 4 Legend:

Table 4 shows the top 10 paired biomarker levels for unfavourable outcome prediction, the optimal prognostic threshold for their ‘varying marker’, AUCm, specificity and sensitivity.

Table 5: Area under each Multivariate ROC curves (AUCm) using original ‘screening thresholds’, and the original threshold levels plus and minus 20%.

Screening marker	Varying marker	AUCm (Original screening threshold)	AUCm (Original screening threshold plus 20%)	AUCm (Original screening threshold minus 20%)
S100b	L-selectin	0.98	0.94	0.94
S100b	IL-6	0.98	0.94	0.88
S100b	NSE	0.97	0.96	0.89
IL-6	L-Selectin	0.96	0.95	0.95
NSE	L-selectin	0.95	0.93	0.93
NSE	S100b	0.94	0.93	0.84
IL-6	IL-8	0.93	0.91	0.89
S100b	IL-8	0.93	0.90	0.89
IL-8	L-selectin	0.92	0.90	0.89
L-selectin	IL-8	0.92	0.91	0.90
L-selectin	S100b	0.92	0.88	0.87
IL-6	NSE	0.91	0.87	0.78
IL-8	IL-6	0.91	0.89	0.89
NSE	IL-8	0.90	0.88	0.88
IL-6	S100b	0.90	0.89	0.89
IL-8	S100b	0.89	0.84	0.82
NSE	IL-6	0.88	0.86	0.83
IL-8	NSE	0.88	0.84	0.80
L-selectin	IL-6	0.88	0.86	0.86
L-selectin	NSE	0.83	0.83	0.75

Table 5 Legend:

Table 5 demonstrates that the original ‘screening thresholds’ of each prognostic paired biomarker levels offered the highest outcome predictive values for unfavourable outcome.

Figure 1:

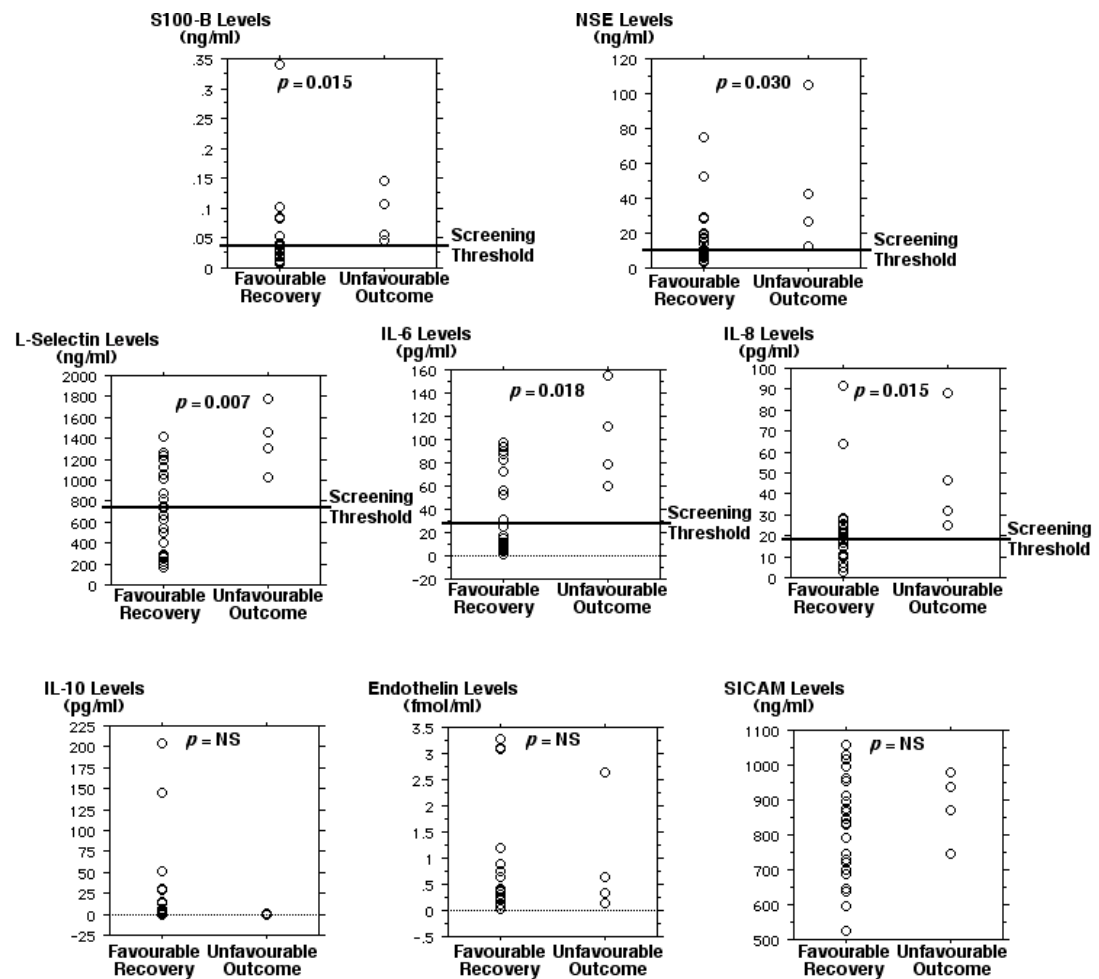


Figure 1 Legend:

Figure 1 shows the day 1 biomarker levels that were significantly different between patients with favourable and unfavourable outcome. Screening thresholds were 0.04 ng/ml for S100b, 12 ng/ml for NSE, 700 pg/ml for L-selectin, 30 pg/ml for IL-6, and 20 pg/ml for IL-8.

Figure 2:

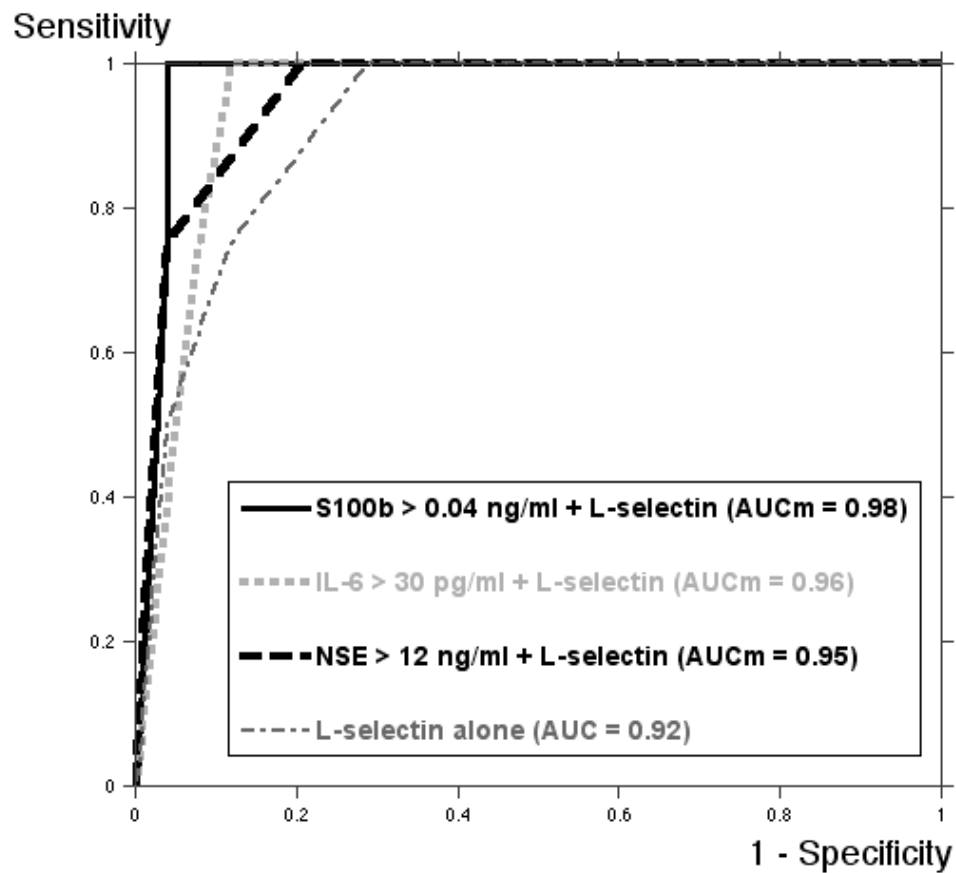


Figure 2 Legend:

Figure 2 shows that the specificity and sensitivity for predicting unfavourable outcome improved when L-selectin was paired with another biomarker. The highest outcome predictive value for unfavourable outcome was achieved when L-selectin was paired with S100b.